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L'évolution de la vie sociale chez les insectes

Joël Meunier

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UNIVERSITÉ FRANÇOIS-RABELAIS DE TOURS

Année Universitaire 2017-2018

HABILITATION A DIRIGER DES RECHERCHES

Discipline : Sciences de la Vie

L'EVOLUTION DE LA VIE SOCIALE CHEZ LES INSECTES

présentée et soutenue publiquement par :

Joël Meunier

le mercredi 4 octobre 2017

Composition du jury :

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M. Raphaël BOULAY	Professeur des Universités	Université de Tours
Mme Christine BRAQUART-VARNIER	Professeur des Universités	Université de Poitiers
Mme Marie CHARPENTIER	Directeur de Recherche, CNRS	Université de Montpellier
M. Thibaud MONNIN	Directeur de Recherche, CNRS	Université de Paris VI
M. Yannick MORET	Directeur de Recherche, CNRS	Université de Dijon

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Je voudrais d'abord remercier (par ordre chronologique) Jacob Koella, Michel Chapuisat, Laurent Keller, Mathias Kölliker et Susanne Foitzik. Ce fut un immense privilège de travailler à vos côtés. Vous m'avez appris la rigueur, la résilience, la remise en question et l'indépendance dans la recherche, mais aussi l'importance de la communication, de l'écoute et du plaisir de développer une approche scientifique commune au sein d'un groupe. J'espère être un jour à la hauteur de ce que vous m'avez apporté et pouvoir transmettre à mon tour le meilleur de ces valeurs. Merci aussi à Anne-Geneviève Bagnères pour m'avoir accueilli dans son équipe à Tours et à David Giron (ainsi qu'à tous les membres de l'IRBI) pour avoir fait en sorte que mon intégration à l'IRBI se fasse de façon on-ne-peut-plus efficace et conviviale.

Mes remerciements s'adressent ensuite à toutes les étudiantes et tous les étudiants que j'ai eu la chance d'encadrer et qui sont à l'origine de la plupart des résultats présentés dans ce mémoire. J'ai eu énormément de chance de faire votre connaissance et de vous avoir avec moi. La motivation, la passion et le dévouement dont vous avez fait preuve au quotidien ont toujours été une source d'énergie incroyable pour moi. Vous avez toutes et tous été les moteurs d'une équipe chaleureuse et efficace, où les échanges formels et informels faisaient souvent oublier le côté routinier et difficile de la recherche pour ne laisser que le plaisir d'être ensemble et l'envie de s'amuser tout en progressant. Je suis très fier de tout ce que vous avez réalisé avec moi, impatient de voir ce que vous allez accomplir dans les prochaines années et plus simplement heureux d'avoir pu partager un moment de science avec vous.

Je voudrais ensuite remercier tous les collègues et amis qui ont jalonné mon parcours à Montpellier, Paris, Lausanne, Bâle, Mayence et maintenant Tours. Vous avez toutes et tous rythmé mon quotidien et partagé de nombreuses étapes importantes de ma vie. Grâce à vous, mon « travail » a toujours été associé au plaisir et au partage, à la convivialité et à la franchise, au respect et à l'amitié. C'est aussi grâce à vous que je peux aujourd'hui présenter ce mémoire.

Pour terminer, il est clair que ma vie professionnelle ne serait rien sans le soutien inaltérable que j'ai eu la chance de trouver dans ma vie personnelle. Un grand merci d'abord à mes parents : vous avez toujours été d'un grand soutien, fait preuve d'un optimisme légendaire et avez réussi à m'accompagner de façon judicieuse et réfléchie dans les méandres d'une carrière académique qui vous était souvent étrangère. Un immense merci enfin à Valérie, et à nos deux fils, Antoine et Eliott, pour leur accompagnement quotidien. Valérie, merci d'avoir toujours cru en moi et d'avoir accepté mon besoin récurrent de nouveaux défis, merci d'avoir supporté l'absence répété de vacances et les montées de stress régulières, merci d'avoir tout abandonné plusieurs fois pour me suivre dans de nouvelles aventures professionnelles, merci enfin pour ton oreille attentive au quotidien et pour ton intérêt constant dans ce qu'il faut bien appeler une passion dévorante. Rien de ce qui est présenté ici n'aurait été possible sans toi.

I. RAPPORT D'ACTIVITES

a) Informations générales

Joël Meunier, né à Montpellier (34) le 26 Juillet 1982. Marié. 2 enfants.

Nationalité : Français

Situation professionnelle : Chargé de Recherche (CR1) au CNRS

Adresse professionnelle : Institut de Recherche sur la Biologie de l'Insecte - UMR 7261
CNRS / Université François-Rabelais
Avenue Monge, Parc Grandmont, 37200 Tours

Contacts : Email : joel.meunier@univ-tours.fr
Téléphone : 02 47 36 73 72 | Fax : 02 47 36 69 66
Internet : www.joelmeunier.wix.com/researchpage

b) Parcours et diplômes

Depuis 2016 **Chargé de Recherche CNRS (CR1 – Recrutement section 29)**
Institut de Recherche sur la Biologie de l'Insecte, UMR 7261, CNRS, Université de Tours
Thématique : *L'évolution de la vie sociale chez les insectes*

2012-16 **Professeur Assistant** dans le département de Susanne Foitzik
Université Johannes-Gutenberg de Mainz, Institut de zoologie, Allemagne
Thématique : *L'évolution de la vie sociale chez les insectes*

2009-12 **Post-doctorant** dans l'équipe de Mathias Kölliker
Université de Bâle, Institut de zoologie & évolution, Suisse
Thématique : *Conflits parents-enfants chez le forficule européen*

2005-09 **Doctorat** ès Sciences de la vie sous la direction de Michel Chapuisat
Université de Lausanne, Département d'écologie & évolution, Suisse
Thèse : *Conflict resolution and evolution of social structures in insect societies*

2004-05 **Master 2 Recherche** Ecologie, Biodiversité et Evolution
Co-habilitation Universités Paris VI & Paris XI, INA-PG et ENS, France
Superviseur de stage : Jacob Koella, Laboratoire de parasitologie évolutive, Université Pierre et Marie Curie, Paris

2000-04 **DEUG, Licence** et **Maitrise** Biologie des Populations et des Ecosystèmes
Université Montpellier II, France
Superviseur de stage : Laurent Dormont, Laboratoire de Zoogéographie, CEFE, Université Montpellier III

c) Responsabilités

Chef de groupe (*Professeur assistant* - Université de Mainz, de 2012 à 2016)

Composition de mon équipe entre 2012 et 2016 : 1 post-doctorant, 2 doctorants, 31 étudiants en Master (stages de 2 à 8 mois), 1 technicienne (à 25%) et 4 aide-animaliers (4h à 10h par semaine).

Comité éditorial de journaux scientifiques

- Membre du comité éditorial du journal *Biology Letters* (depuis 2017).
- Review editor pour le journal *Frontiers in Ecology and Evolution - Social evolution* (depuis 2016)
- Editeur invité du journal *Current Opinion in Insect Sciences* (numéro spécial prévu pour 2018).
- Faculty member de la *F1000* - section *Behavioural ecology* (depuis 2015).

Evaluation de >100 manuscrits pour >35 journaux internationaux

Par exemple : *American Naturalist, Animal Behaviour, Behavioral Ecology, Behavioral Ecology and Sociobiology, Biological Reviews, Biology Letters, BMC Evolutionary Biology, eLife, Evolution, Journal of Evolutionary Biology, Nature Communications, Philosophical Transactions of the Royal Society B: Biological Sciences, PNAS* et *Proceedings of the Royal Society B: Biological Sciences*. Plus de détails sur https://publons.com/author/399987.

Evaluation de projets de recherche

- Agence Nationale de la Recherche (ANR, France) – Membre d'un Comité d'Evaluation Scientifique.
- Deutsches Forschungsgemeinschaft (DFG, Allemagne)
- National Science Foundation (NSF, Etats-Unis)
- Natural Environment Research Council (NERC, UK)

Evaluation de doctorats

- Membre de comité / jury de thèse en France :
  1. Adrien Le Navenant (2017). Encadrante : Magalie Rault-Léonardon. Univ Avignon.
  2. Caroline Michaud (2017). Encadrants : Franck Dedeine et Géraldine Dubreuil. Univ Tours.
  3. Charly Jehan (2017). Encadrants : Yannick Moret et Thierry Rigaud. Univ Dijon.
  4. Sylvine Durand (2017). Encadrants : Christine Braquart-Varnier et Sophie Beltran-Bech. Univ Poitiers.
  5. Diane Bigot (2016). Encadrants : Elisabeth Herniou et Philippe Gayral. Univ Tours.
- Rapporteur de jury de thèse à l'étranger :
  1. Peterson Coates (2017). Encadrants : Adam Stow et Tom Chapman. Univ Macquarie, Sydney, Australie.
  2. Amaranta Fontcuberta (2017). Encadrant : Michel Chapuisat. Univ Lausanne, Suisse.
  3. Andres Arce (2013). Encadrants : Daniel Rozen et Per Smiseth. Univ Manchester, Royaume-Uni.

Responsabilités collectives

- Co-organisateur des séminaires inter/intra département (Université de Tours, 2017)
- Organisateur de séminaires inter-départements (Université de Mainz, 2012 - 2015)
- Organisateur des séminaires du département d'évolution (Université de Mainz, 2012 - 2016)
- Webmaster du site du département (Université de Mainz, 2012 - 2015)
- Webmaster du forum pour étudiants de la filière EBE Paris (2004 - 2013)
- Co-organisateur d'un Journal club (Université de Bâle, 2011-12)
- Représentant élu du corps intermédiaire de la faculté de biologie et de médecine de l'université de Lausanne (2007 - 2009).

d) Enseignements

Résumé : *Depuis 2006, j'ai enseigné un total de 570 heures, réparties entre des travaux pratiques, des travaux dirigés et des cours magistraux. Ces enseignements ont été faits en français et en anglais.*

Ecologie comportementale

Cours magistraux, TD et TP | 2008, 2012-17 *Total = 190h*

Utilisant une approche thématique et des exemples précis, ce cours permet aux étudiants de se familiariser aux concepts de l'éthologie et de l'écologie comportementale, avec un focus particulier sur les invertébrés. Les travaux pratiques consistaient en la réalisation d'analyses de comportements.

Design expérimental

Cours magistraux, TD et TP | 2008-17 *Total = 160h*

L'objectif de ce module est d'apprendre aux étudiants à avoir une démarche de recherche scientifique en leur donnant d'abord les clés pour réaliser une expérience dans de bonnes conditions (cours) et ensuite en leur demandant de réaliser leur propre travail de recherche sur une courte période (TP). Pour ce dernier point, les étudiants doivent définir une question scientifique nouvelle à partir de la littérature, déterminer les hypothèses qu'ils souhaitent tester, mettre en place un design expérimental, réaliser l'expérience, analyser leurs données, rédiger un rapport sous forme d'article et présenter les résultats à l'oral. Suivant l'endroit où j'ai effectué ces enseignements, la partie TP était fortement réduite et n'impliquait qu'une approche « théorique ».

Bio-statistiques

Cours magistraux, TD et TP | 2009-15 *Total = 90h*

Ces cours, TP et TD ont eu pour but à la fois d'enseigner les analyses statistiques généralement utilisées en biologie et d'apprendre aux étudiants à utiliser un logiciel de statistique et de programmation libre de droit, le logiciel R. Les étudiants sont amenés à travailler sur des jeux de données réels issus des recherches du Département afin de les familiariser avec le travail quotidien d'un chercheur.

Zoologie

Cours magistraux, TD et TP | 2012-15 *Total = 60h*

Il s'agit ici d'enseigner aux étudiants les bases permettant d'identifier le nom de différentes espèces animales (mes enseignements sont focalisés sur les insectes), ainsi que de les familiariser avec les notions de systématique et d'évolution des traits d'histoire de vie.

Présentation scientifique (Soft skills)

Cours magistraux, TD et TP | 20013-15 *Total = 40h*

Lors de ce module, les étudiants reçoivent un enseignement théorique sur les règles de base de la présentation scientifique, la recherche bibliographique, l'utilisation d'un logiciel de référencement (Mendeley desktop) et sur les bases de l'écriture d'un article scientifique (en anglais). Ils doivent ensuite réaliser un poster à partir d'une publication scientifique de leur choix et en faire un PowerPoint qu'ils présentent en 10 minutes.

Ecologie chimique

Cours magistraux, TD et TP | 2010 *Total = 30h*

Les étudiants suivant ce module reçoivent une formation théorique et pratique concernant l'écologie chimique, son importance dans la régulation du comportement animal et sur comment analyser les résultats de GC-MS. Mes cours magistraux et TP sont principalement centrés sur les types d'analyses statistiques à conduire avec ces jeux de données.

e) Contrats de recherche obtenus

2014 Research Grant Program (200 k€)
Deutsche Forschungsgemeinschaft (DFG, Allemagne)
Projet : *Importance of social immune system on the early evolution of social life*

2013 Inneruniversitäre Forschungsförderung (15 k€)
Université de Mainz (Allemagne)
Projet : *The expression of immune responses in families of the European earwigs F. auricularia*

2013 Research Grant Program (184k€)
Deutsche Forschungsgemeinschaft (DFG, Allemagne)
Projet : *Influence of ecology and social interactions on the early evolution of family life*

f) Communications orales et affichées

Séminaires invités

11. Dpt d'Ecologie et d'Evolution, Université de Lausanne, Suisse (05.2017)
10. Dpt of Evolutionary Biology and Environmental Studies, Université de Zurich, Suisse (11.2016)
09. Institut de Recherche sur la Biologie de l'Insecte (IRBI), Université de Tours, France (09.2014)
08. Institut de Systématique, Evolution et Biodiversité (ISYEB), MNHN Paris, France (06.2014)
07. Laboratoire Ecologie et Evolution, Université Pierre et Marie Curie, Paris, France (02.2014)
06. Laboratoire Biogéosciences, Université de Dijon, Dijon, France (02.2014)
05. Division of Evolutionary Ecology & Behavioral Ecology, Université de Bern, Suisse (11.2012)
04. Institute of Zoology and Evolution, JG Université de Mainz, Allemagne (10.2011)
03. Institut de Recherche sur la Biologie de l'Insecte (IRBI), Université de Tours, France (04.2011)
02. Centre de Recherche sur la Cognition Animale (CRCA), Univ. de Toulouse, France (02.2011)
01. Institute of Zoology and Evolution, Université de Bâle, Suisse (04.2009)

Communications dans des congrès scientifiques

23. Meunier J, 04.2017. **Présentation orale invitée**.
Contrasting effects of maternal deprivation in an insect
ReproSciences 2017 (GdR Repro), Tours, France.
22. Meunier J, 03.2016. Présentation orale.
Friend or Foe? Costs and benefits of gregarine infection in the European earwigs.
Réseaux Ecologiques des Interactions Durables (REID), Poitiers, France.
21. Meunier J, 08.2015. Présentation orale.
A transient stress of social isolation determines resistance against pathogen infection in a group-living insect.
Union for the Study of Social Insects – Section française (IUSSI), Tours, France.
20. Meunier J, 09.2014. Présentation orale.
Frass production provides anti-microbial protections in an insect with maternal care
107th Annual Meeting of the German Zoological Society (DZG), Göttingen, Allemagne.
19. Meunier J, 05.2014. Présentation orale.
Frass production provides antimicrobial protection in an insect with maternal care
Colloque sur l'immunité des invertébrés (ImmunInv2014), Dijon, France.
18. Meunier J, 02.2014. Présentation orale.
The determinants of offspring quantity & quality at egg hatching
1st Earwig Science Symposium (ESS), Bâle, Suisse.

17. Meunier J and Kölliker M, 09.2013. Présentation orale.
Inbreeding depression in an insect with maternal care: family interactions, life-stage and offspring sex.
106th Annual Meeting of the German Zoological Society (DZG), Munich, Allemagne.
16. Meunier J and Kölliker M, 08.2013. Présentation orale.
Influence of entangled social conflicts on family life in a subsocial insect.
Union for the Study of Social Insects – Section française (IUSSI), Villetaneuse, France.
15. Meunier J and Kölliker M, 02.2012. Présentation orale.
Parental antagonism and parent-offspring coadaptation interact to shape family life.
Swiss Botanical, Mycological and Zoological Societies Meeting (Biology12), Fribourg, Suisse.
14. Meunier J and Kölliker M, 08.2011. Poster.
Family life, genetic conflicts and co-adaptation in earwigs.
13th European Congress for Evolutionary Biology (ESEB), Tubingen, Allemagne.
13. Meunier J and Kölliker M, 04.2011. Présentation orale.
Good or bad mothers? Low resource availability suppresses benefits of maternal care in the European earwig.
Union for the Study of Social Insects – Section française (IUSSI), Banyuls-sur-mer, France.
12. Meunier J and Kölliker M, 02.2011. Poster. **# Prix Poster #**
Good or bad mothers? Low resource availability suppresses benefits of maternal care in the European earwig.
Swiss Botanical, Mycological and Zoological Societies Meeting (Biology11), Zürich, Suisse.
11. Meunier J, Delemont O and Lucas C, 10.2010. Présentation orale.
Recognition in ants: social origin matters.
Colloque de Biologie de l'Insecte (CBI), Lyon, France.
10. Meunier J and Kölliker M, 02.2010. Poster. **# Prix Poster #**
One clutch or two clutches? Alternative reproductive strategies in the European earwig *Forficula auricularia*.
Swiss Botanical, Mycological and Zoological Societies Meeting (Biology10), Neuchatel, Suisse.
09. Meunier J, Delaplace L and Chapuisat M, 09.2009. Poster.
Reproductive conflicts and egg recognition in socially polymorphic ants.
12th European Congress for Evolutionary Biology (ESEB), Turin, Italie.
08. Meunier J, Delaplace L and Chapuisat M, 02.2009. Présentation orale.
Egg discrimination by workers in the ant *Formica selysi*.
Swiss Botanical, Mycological and Zoological Societies Meeting (Biology09), Bern, Suisse.
07. Meunier J, Delaplace L and Chapuisat M, 02.2009. Présentation orale.
Queen number influences egg recognition in the ant *Formica selysi*.
PhD Students meeting of the Faculty of Biology and Medicine (D-day), Lausanne, Suisse.
06. Meunier J, Delaplace L and Chapuisat M, 09.2008. Présentation orale.
Egg recognition in monogyne and polygyne colonies of the ant *Formica selysi*.
Symposium Ecology and Evolution (Seeds), Lausanne, Suisse.
05. Meunier J and Chapuisat M, 08.2008. Présentation orale.
The inheritance of queen size and queen number in ants.
Union for the Study of Social Insects – European meeting. (IUSSI), La Roche-en-Ardenne, Belgique.
04. Meunier J and Chapuisat M, 08.2007. Poster. **#Prix Poster#**
The inheritance of queen size and queen number in ants.
Union for the Study of Social Insects – Section française (IUSSI), Toulouse, France.

03. Meunier J and Chapuisat M, 03.2007. Présentation orale.
The inheritance of queen size and queen number in ants.
3rd meeting in Ecology and Behavior (SERL), Montpellier, France.

02. Meunier J and Chapuisat M, 02.2007. Présentation orale.
The inheritance of queen size and queen number in ants.
Swiss Botanical, Mycological and Zoological Societies meeting (Biology07), Zürich, Suisse.

01. Meunier J, West SA and Chapuisat M, 08.2006. Poster.
Sex ratio adjustment in response to relatedness asymmetry variation in social hymenoptera: a meta-analysis.
Union for the Study of Social Insects – International meeting (IUSSI), Washington DC, USA.

Communications grand public

2017 - *Le monde des perce-oreilles*. Animation à l'école maternelle des Gués, Veigné, France
2014 - *Insectes utiles ou parasites ?* Interview dans un documentaire télévisuel (X:enius) sur Arte.
- *How I learned to love the evil-looking earwig*. Interview pour le magazine *The week*.
2012 - *Le génie des fourmis*. Conférence publique. Muséum d'Histoire Naturelle de Colmar, France.
2010 - *Unbarmherzige Ohrwurmmütter*. Interview dans le Basler Zeitung, Suisse.
- *Earwigs & Evolution*. 550ème anniversaire de l'université de Bâle.
2008 - *Le meilleur ami des fourmis*. Participation à un documentaire TV, TSR, Suisse.
- *L'organisation sociale des fourmis*. Journées portes ouvertes de l'université de Lausanne, Suisse.
2007 - *Des fourmis à la technologie*. Innovation technologiques, ADIRA & OSLO-Software, Lyon.
- *Le monde des fourmis*. Journées portes ouvertes de l'université de Lausanne, Suisse.
2006 - *Biologie et fourmis*. Journées portes ouvertes de l'université de Lausanne, Suisse.

g) Encadrement de la recherche

Résumé : *Depuis 2006, j'ai encadré 1 projet de post-doctorat, 3 projets de doctorat, 22 projets de Master 2 et 16 projets de Master 1. Les noms soulignés indiquent que le travail de l'étudiant a donné lieu à au moins une publication scientifique.*

Post-doctorant :

- Fanny Vogelweith (11.2015 - 11.2017). Encadrement à 100%. Université de Mainz.
  Financement : Humboldt Research Fellowship (obtenu par la post-doctorante).

Etudiants en doctorat *:*

- Sophie Van Meyel (depuis 10.2017). Encadrement à 50% (David Giron). Université de Tours.
  Financement : Ecole doctorale SSBCV, Tours
- Maximilian Körner (depuis 10.2014). Encadrement à 100%. Université de Mainz.
  Financement : DFG, voir contrats de recherche obtenus - section (e)
- Jos Kramer (05.2013 - 01.2017). Encadrement à 100%. Université de Mainz.
  Financement : DFG, voir contrats de recherche obtenus - section (e)

Etudiants en Master 2 ou équivalent étranger (durée du stage et année) :

- Audrey Fournier (6 mois, 2017). Encadrement à 100%. IRBI, Université de Tours.
  Effet du challenge immunitaire sur les soins maternels chez le forficule européen.

- Armin Joos (8 mois, 2016). Encadrement à 100%. Université de Mainz.
  Transgenerational immune priming and feces as an active mechanism of social immunity in earwigs
- Janina Diehl (8 mois, 2016). Encadrement à 100%. Université de Mainz.
  Social immunity in Forficula auricularia
- Francisco Arcila (8 mois, 2016). Encadrement à 100%. Université de Mainz.
  Unraveling the nature of Gregarina ovata and Forficula auricularia co-existence.
- Julia Meyer (8 mois, 2015). Encadrement à 100%. Université de Mainz.
  Feces and pathogen defense in the European earwig, Forficula auricularia.
- Tom Ratz (8 mois, 2015). Encadrement à 100%. Université de Mainz.
  The influence of juvenile's cohort on life-history traits is population-specific in earwigs.
- Janina Diehl (4 mois, 2014). Encadrement à 100%. Université de Mainz.
  The role of frass in nymphs interactions of the European earwig.
- Philip Kohlmeier (8 mois, 2014). Encadrement à 100%. Université de Mainz.
  How host-pathogen interactions shape basic forms or social life in insects
- Maximilian Körner (8 mois, 2014). Encadrement à 100%. Université de Mainz.
  The role of frass sharing in pathogen defense of the European earwig.
- Christine Scheiner (4 mois, 2014). Encadrement à 100%. Université de Mainz.
  Long-term effects of orphaning on behavior and mate choice in an insect with facultative maternal care
- Julia Thesing (8 mois, 2014). Encadrement à 100%. Université de Mainz.
  Long-term effects of maternal care in the European earwig Forficula auricularia.
- Kai Höllander (4 mois, 2013). Encadrement à 100%. Université de Mainz.
  Isolation in the European earwig: benefit or stress?
- Lisa Koch (8 mois, 2013). Encadrement à 100%. Université de Mainz.
  Immunity in animal species with facultative social life
- Charlotte Weiß (4 mois, 2013). Encadrement à 100%. Université de Mainz.
  Cooperation among adult earwigs.
- Faban Wagner (8 mois, 2013). Encadrement à 50%. Université de Mainz.
  Habitat preferences and diversity of male orchid bees in Cusuco National Park.
- Laura Müller (4 mois, 2012). Encadrement à 100%. Université de Mainz.
  Influence of queen and brood presence on social organization in the ant Myrmica rubra.
- Laura Nimtz (4 mois, 2012). Encadrement à 50%. Université de Mainz.
  The influence of colors on nest choice in the ants Myrmica rubra and Temnothorax nylanderi.
- Teresa Stehle (4 mois, 2012). Encadrement à 100%. Université de Mainz.
  Orientation in the ant Myrmica rubra after foraging.
- Lina Sandrin (8 mois, 2011). Encadrement à 50%. Université de Basel.
  Determinants of the multiple mating in the European earwig.
- Stefan Boos (8 mois, 2010). Encadrement à 50%. Université de Basel.
  When family starts in a burrow: Why is maternal egg attendance beneficial in earwigs?
- Luma Delaplace (6 mois, 2008). Encadrement à 50%. Université de Lausanne.
  Queen-worker conflict over male production in the ant Formica selysi.

- Benjamin Bricault (6 mois, 2006). Encadrement à 50%. Université de Lausanne.
  Colony conflict over sex-ratio in the ant Formica selysi.

Etudiants en Master 1 ou équivalent étranger (durée du stage et année) :

- Cybèle Prigot (1 mois, 2017). Encadrement à 100%. Université de Tours.
  Importance du microbiote dans l'immunité chez le forficule européen.
- Carina Enders (2 mois, 2015). Encadrement à 100%. Université de Mainz.
  On the correlates of Gregarine infection in the European earwig.
- Juliane Hartke (2 mois, 2015). Encadrement à 100%. Université de Mainz.
  Enemy at the gates – Alignment of immune activity in social insects.
- Marina Psalti (2 mois, 2015). Encadrement à 100%. Université de Mainz.
  Influence of social isolation and starvation on the behavior of adult European earwigs.
- Armin Joos (2 mois, 2015). Encadrement à 100%. Université de Mainz.
  Sex-specific immune defense in the European earwig.
- Philipp Sprenger (2 mois, 2015). Encadrement à 100%. Université de Mainz.
  Association between physiological and behavioral immunity in earwigs.
- Teresa Christl (2 mois, 2014). Encadrement à 100%. Université de Mainz.
  Influences of day time and relatedness on maternal care in the European earwig.
- Beatrice Dewenter (2 mois, 2014). Encadrement à 100%. Université de Mainz.
  Influence of male morphology on behavior & immunity in the European earwig.
- Aytül Dadak (2 mois, 2014). Encadrement à 100%. Université de Mainz.
  Influence of relatedness and days after hatching on the expression of maternal care in the European earwig.
- Stephan Fries (2 mois, 2013). Encadrement à 100%. Université de Mainz.
  Food sharing in earwigs: a form of reciprocal altruism?
- René Radke (2 mois, 2013). Encadrement à 100%. Université de Mainz.
  Influence of sex on the dynamic of the immune response in the European earwig.
- Philip Kohlmeier (2 mois, 2013). Encadrement à 100%. Université de Mainz.
  Influence of maternal care, body mass and forceps size on earwig's immunocompetence.
- Maximilian Körner (2 mois, 2013). Encadrement à 100%. Université de Mainz.
  Do relatedness and pathogen presence influence social interactions in the European earwig?
- Julia Thesing (2 mois, 2013). Encadrement à 100%. Université de Mainz.
  Group-living and immune responses in the European earwig Forficula auricularia.
- Julia Post (2 mois, 2013). Encadrement à 100%. Université de Mainz.
  Influence of mold spores on earwig behaviors.
- Joachim Falk (2 mois, 2013). Encadrement à 100%. Université de Mainz.
  Sibling cooperation in the European earwig.

h) Liste de publications

Vue d'ensemble de mes publications

- 35 articles publiés dans des journaux internationaux dont :
  - 28 en tant que premier ou dernier auteur
  - 7 en tant que co-auteur
- 2 chapitres dans des livres grand public

Journal	IF_{2015}	Quartile	Nombre	Année(s)
Nature Communications	11,33	Q1	1	2015
New Phytologist	7,21	Q1	1	2010
Phil. Transac. of the Royal Society B: Biol sciences	5,85	Q1	1	2015
Functional Ecology	5,21	Q1	1	2017
Proc. of the Royal Society B: Biol sciences	4,82	Q1	3	2012, 2014, 2015
BMC Evolutionary Biology	3,41	Q2	3	2014, 2015, 2017
American Naturalist	3,15	Q2	1	2014
Animal Behaviour	3,17	Q1	2	2010, 2011
PloS ONE	3,06	Q1	1	2011
Behavioral Ecology	3,03	Q1	4	2008, 2014, 2016, 2016
Oecologia	2,90	Q2	1	2016
Biology letters	2,82	Q1	1	2012
Journal of Evolutionary Biology	2,75	Q2	4	2009, 2013, 2015, 2016
Behavioral Ecology and Sociobiology	2,38	Q1	2	2010, 2011
Journal of Insect Physiology	2,27	Q1	1	2015
Evolutionary Ecology	1,88	Q2	1	2012
Ethology	1,72	Q2	1	2014, 2015
Ecological Entomology	1,69	Q1	2	2013, 2015
Insectes sociaux	1,27	Q2	1	2008
Chimia	1,00	Q3	1	2011
F1000 Research	NA	NA	1	2016
Communicative & Integrative biology	NA	NA	1	2011

Liste des publications

Les noms soulignés indiquent les étudiants/post-doctorants sous ma supervision.

35. Kramer J, Körner M, Diehl JMC, Scheiner C, Yüksel-Dadak A, Christl T, Kohlmeier P and **Meunier J** (2017). When earwig mothers do not care to share: parent-offspring competition and the evolution of family life. *Functional Ecology*, in press. (DOI: 10.1111/1365-2435.12915)

34. Vogelweith F*, Körner M*, Foitzik S and **Meunier J** (2017). Age, pathogen exposure, but not maternal care shape offspring immunity in an insect with facultative family life. *BMC Evolutionary Biology,* 17:69. [*co-premier auteurs]

33. Körner M, Diehl JMC and **Meunier J** (2016). Growing up with feces: benefits of allo-coprophagy in families of the European earwig. *Behavioral Ecology*, 27 (6):1775-1781.

32. Ratz T, Kramer J, Veuille M and **Meunier J** (2016). The population determines whether and how life-history traits vary between reproductive events in an insect with maternal care. *Oecologia*, 182, 443–452.

31. Kohlmeier P, Holländer K and **Meunier J** (2016). Survival after pathogen exposure in group-living insects: don't forget the cost of social isolation! *Journal of Evolutionary Biology*, 29, 1867–1872.

30. Kramer J and **Meunier J** (2016). Kin and multilevel selection in social evolution: a never-ending controversy? *F1000 Research*, 5, 577.

29. Kramer J and **Meunier J** (2016) Maternal condition determines offspring behavior towards family members in the European earwig. *Behavioral Ecology*, 27 (2):494-500.

28. Kohlmeier P, Dreyer H and **Meunier J** (2015) PO-CALC: A novel tool to correct common inconsistencies in the measurement of phenoloxidase activities. *Journal of Insect Physiology,* 75 :80-84.

27. Thesing J*, Kramer J*, Koch LK and **Meunier J (**2015) Short-term benefits, but transgenerational costs of maternal loss in an insect with facultative maternal care. *Proceedings of the Royal Society B: Biological Sciences*, 282(1817), 20151617 [*co-premier auteurs]

26. Kramer J, Thesing J and **Meunier J** (2015) Negative association between parental care and sibling cooperation in earwigs: a new perspective on the early evolution of family life? *Journal of Evolutionary Biology*, 28, 1299–1308.

25. Kölliker M, Boos S, Wong JWY, Röllin L, Stucki D, Raveh S, Wu M and **Meunier J** (2015) Parent-offspring conflict and the genetic trade-offs shaping parental investment. *Nature Communications*, 6, 6850.

24. Diehl JMC, Körner M, Pietsch M and **Meunier J** (2015) Feces production as a form of social immunity in an insect with facultative maternal care. *BMC Evolutionary Biology*, 15, 40.

23. **Meunier J** (2015) Social immunity and the evolution of group-living in insects. *Philosophical Transactions of the Royal Society B: biological sciences*, 370, 20140102.

22. Sandrin L, **Meunier J,** Raveh S, Walser JC and Kölliker M (2015) Multiple paternity and mating group size in the European earwig, *Forficula auricularia*. *Ecological entomology*, 40(2), 159–166.

21. Wong JWY*, **Meunier J***, Lucas C and Kölliker M (2014) Paternal signature in kin recognition cues of a social insect: Concealed in juveniles, revealed in adults. *Proceedings of the Royal Society of London B: biological Sciences*, 281: 20141236. [*Authors contributed equally to the work]

20. Koch LK and **Meunier J** (2014) Mother and offspring fitness in an insect with maternal care: phenotypic trade-offs between egg number, egg mass and egg care. *BMC Evolutionary Biology*, 14(1):125.

19. Weiss C, Kramer J, Holländer K and **Meunier J** (2014) Influences of relatedness, food deprivation and sex on adult behaviors in the group-living insect *Forficula auricularia. Ethology*, 120, 923-932.

18. Boos S, **Meunier J**, Pichon S and Kölliker M (2014) Maternal care provides anti-fungal protection to eggs in the European earwig. *Behavioral Ecology*, 25(4), 754-761.

17. Falk J, Wong JWY, Kölliker M and **Meunier J** (2014) Sibling cooperation in earwig families provides insights into the early evolution of social life. *The American Naturalist*, 183(4), 547-557.

16. **Meunier J** and Kölliker M (2013) Inbreeding depression in an insect with maternal care: influences of family interactions, life-stage and offspring sex. *Journal of Evolutionary Biology*, 26(10), 2209-20.

15. Wong JWY, **Meunier J** and Kölliker M. (2013) The evolution of parental care in insects: The roles of ecology, life history and the social environment. *Ecological entomology*, 38(2), 123-137.

14. **Meunier J** and Kölliker M (2012) Parental antagonism and parent-offspring co-adaptation interact to shape family life. *Proceedings of the Royal Society of London B: biological Sciences* 279, 3981-3988.

13. **Meunier J** and Kölliker M (2012) When it is costly to have a caring mother: food limitation erases the benefits of parental care in earwigs. *Biology letters* 8(4), 547-550.

12. **Meunier J**, Wong JWY, Gomez Y, Kuttler S, Röllin L, Stucki D and Kölliker M (2012) One clutch or two clutches? Fitness correlates of coexisting alternative female life-histories in the European earwig. *Evolutionary Ecology* 26(3), 669-682.

11. Mas F, **Meunier J** and Kölliker M (2011) A new function of hydrocarbons in insect communication: maternal care and offspring signalling in the European earwig. *Chimia* 65:9, 744.

10. **Meunier J** (2011) Can multiple pathways mediate the influence of queen number on nestmate discrimination in ants? *Communicative and Integrative Biology* 4(5), 609-611.

09. **Meunier J**, Delémont O and Lucas C (2011) Recognition in ants: social origin matters. *PLoS ONE* 6(5): e19347.

08. **Meunier J***, Fingueiredo Pinto S*, Burri R and Roulin A (2011) Eumelanin-based coloration and fitness parameters in birds: a meta-analysis. *Behavioral Ecology and Sociobiology* 65, 559-567. [*co-premier auteurs]

07. **Meunier J**, Reber A and Chapuisat M (2011) Queen acceptance in a socially polymorphic ant. *Animal Behaviour* 81, 163-168.

06. Masclaux F, Hammond R, **Meunier J**, Gouhier-Darimont C, Keller L and Reymond P (2010) Competitive ability not kinship affects growth of *Arabidopsis thaliana* accessions. *New Phytologist* 185(1), 322-331.

05. **Meunier J**, Delaplace L and Chapuisat M (2010) Reproductive conflicts and egg discrimination in a socially polymorphic ant. *Behavioral Ecology and Sociobiology* 64(10), 1655-1663.

04. Reber A, **Meunier J** and Chapuisat M (2010) Flexible colony founding strategies in a socially polymorphic ant. *Animal Behaviour* 78, 467-472.

03. **Meunier J** and Chapuisat M (2009) The determinants of queen size in a socially polymorphic ant. *Journal of Evolutionary Biology* 22, 1906-1913.

02. Holzer B, **Meunier J**, Keller L and Chapuisat M (2008) Stay or drift? Queen acceptance in the ant *Formica paralugubris*. *Insectes sociaux* 55, 392-396.

01. **Meunier J**, West SA and Chapuisat M (2008) Split sex ratios in the social Hymenoptera: a meta-analysis. *Behavioral Ecology* 19, 382-390.

Chapitres de livre grand public

02. **Meunier J** (2012). Odeurs et interactions familiales. Dans le livre "Ecologie chimique : le langage de la nature". Editions Cherche midi, p 80.

01. Darrouzet E, **Meunier J**, Bagnères AG & Schatz B (2011). Le génie des insectes sociaux. Dans le livre "Le génie de la nature". Editions Biotope, p 86- 111.

II. MEMOIRE

1. INTRODUCTION GENERALE

La vie de groupe est un phénomène commun dans la nature dont la complexité s'étend des simples groupes temporaires formés par l'attraction mutuelle entre individus jusqu'aux sociétés complexes et permanentes, structurées par la division du travail (Wilson, 1971; Costa, 2006; Aron, 2007) (Tableau 1). Le succès écologique des espèces vivant en groupe repose principalement sur les bénéfices directs et indirects que la vie sociale et ses formes de coopérations apportent à ses membres (Hamilton, 1964; Wilson, 1975; West *et al.*, 2007; Abbot *et al.*, 2011; Bourke, 2014). La vie sociale est par exemple connue pour améliorer la survie du couvain, pour augmenter l'efficacité de recherche et d'acquisition de nourriture ou encore pour mieux protéger les individus contre les prédateurs (Krause & Ruxton, 2002b). Pour autant, le groupe n'offre pas uniquement un cadre de vie harmonieux et peut être la source de coûts très importants pour ses membres. Ces coûts viennent d'une part des conflits sociaux qui émergent, par exemple, lorsque les individus entrent en compétition pour l'accès à une ressource limitée et/ou à la reproduction (Ratnieks *et al.*, 2006; Meunier *et al.*, 2008, 2010; East & Hofer, 2010; Meunier & Kölliker, 2012a). D'autre part, ces coûts viennent du risque inhérent et accru d'infection par des pathogènes lorsque l'on vit en groupe (Schmid-Hempel, 1998; Cremer *et al.*, 2007). Ce risque repose principalement sur les fréquents contacts entre individus qui facilitent la transmission de parasites et sur le fort apparentement génétique qui rend ses membres susceptibles aux mêmes types de pathogènes (Schmid-Hempel, 1998; Pie *et al.*, 2004; Cremer *et al.*, 2007; Stroeymeyt *et al.*, 2014; Meunier, 2015). Comprendre l'émergence et le maintien de la vie de groupe nécessite donc de découvrir et d'étudier les mécanismes évolutifs (qu'ils soient au niveau du gène, de l'individu ou du groupe ; voir Okasha, 2008; Bourke, 2011; Kramer & Meunier, 2016a) permettant à ses membres de résoudre les conflits sociaux, de limiter les risques d'infection, et plus généralement de leur apporter des bénéfices nets en matière de fitness.

Tableau 1 | Classification des principaux degrés de socialité en fonction de facteurs de complexité croissante. Il est important de noter que cette classification ne permet pas de placer sur une échelle évolutive toutes les formes de vie sociale connues [cette limite dans la classification de la socialité est discutée dans (Costa & Fitzgerald, 1996; Costa, 2006; Bourke, 2011)]. Tableau adapté de (Wilson, 1971; Aron, 2007).

	Interactions entre individus	Soins parentaux	Site d'élevage en commun	Coopération dans les soins aux jeunes	Individus spécialisés dans la reproduction
Vie solitaire					
Grégarité	+				
Subsocialité*	+	+			
Colonialité	+	+	+		
Communalité	+	+	+	+	
Eusocialité	+	(+)	(+)	+	+

* Inclut les espèces **précociales** (ancestrales), chez qui les juvéniles peuvent fourrager seuls dès l'éclosion des œufs et donc survivre sans leurs parents, et les espèces **altriciales**, dont les juvéniles sont peu ou pas mobiles et ne peuvent donc pas survivre sans soins parentaux.

A ce jour, un grand nombre d'études théoriques et empiriques se sont intéressées aux coûts et bénéfices des interactions sociales dans des espèces où la vie de groupe est obligatoire et/ou permanente (Schmid-Hempel, 1998; Ratnieks *et al.*, 2006; Hudson & Trillmich, 2007; Davies *et al.*, 2012). C'est le cas par exemple des espèces d'insectes eusociaux comme les fourmis, les termites et quelques abeilles et guêpes (Wilson, 1971), chez qui les individus vivent en groupe du début à la fin de leur vie et pour lesquelles l'isolation sociale conduit souvent à une mort précoce. Il en est de même chez les espèces avec une vie de famille altriciale (= nidicole, Tableau 1), que l'on trouve généralement chez tous les mammifères et une grande majorité des oiseaux (Royle *et al.*, 2012), et pour qui la survie des juvéniles nécessite la vie de famille et en particulier l'expression de soins parentaux. Toutes ces études ont été d'une grande importance dans notre compréhension de l'évolution de la vie sociale, car elles ont permis de révéler la nature des processus limitant les coûts des interactions sociales lorsque les individus ont perdu la capacité à vivre de façon solitaire. Pour autant, se focaliser sur les sociétés eusociales et altriciales pour comprendre l'évolution de la vie sociale présente une limite importante : ces sociétés ne permettent pas d'étudier quels sont les mécanismes responsables de l'émergence et du maintien de la vie de groupe à partir d'un état solitaire (Smiseth *et al.*, 2003; Falk *et al.*, 2014; Meunier, 2015) – une transition considérée comme majeure dans l'évolution de la vie (Szathmary *et al.*, 1995; Bourke, 2011). Cette limite laisse notamment deux questions cruciales en suspens : premièrement, est-ce que les mécanismes connus pour favoriser le maintien de la vie de groupe chez les espèces eusociales et altriciales (par ex. la coopération ou l'immunité sociale) sont des traits secondaires qui ont simplement dérivé de la contrainte évolutive liée à l'obligation de la vie de groupe, ou est-ce qu'ils sont des mécanismes ancestraux présents dans les systèmes sociaux moins dérivés et donc plus généralement, est-ce qu'ils sont des moteurs globaux de l'évolution de la vie sociale ? Deuxièmement, est-ce que d'autres mécanismes favorisant la vie sociale n'opèrent plus dans les systèmes sociaux obligatoires mais restent présents dans ceux plus ancestraux ?

Une opportunité unique de mieux répondre à ces questions et donc de comprendre la transition évolutive entre vie solitaire et vie de groupe est d'étudier les coûts et bénéfices de la vie sociale lorsque cette dernière est facultative – c'est-à-dire lorsque chaque individu a la possibilité de vivre seul ou dans un groupe structuré. Une de ces formes de vie sociale facultative se retrouve dans les espèces présentant une vie de famille précociale (= nidifuge, en opposition à altricial ou nidicole, voir Smiseth *et al.*, 2003; Falk *et al.*, 2014) (Tableau 1).

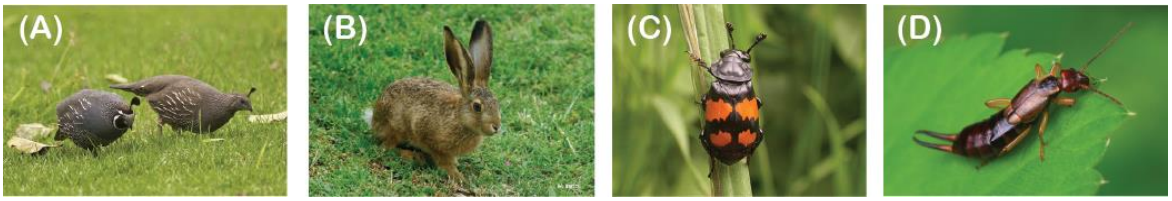

Figure 2 | **Quelques exemples d'espèces précociales, c.à.d. chez qui les juvéniles sont rapidement mobiles et peuvent survivre à l'absence de parents.** (A) Le Colin de Californie *Callipepla californica* (photo: J. Hempel), (B) Le lièvre européen *Lepus europaeus* (photo: M. Bartoli), (C) Le scarabée nécrophore *Nicrophorus vespilloides* (photo: www.eakringbirds.com), (D) Le forficule européen *Forficula auricularia* (photo: D. Scharnowski).

Alors que toutes les formes de vie familiales sont caractérisées par l'expression de soins parentaux envers les juvéniles (et/ou les œufs), les espèces précociales (Figure 2) se différencient des espèces altriciales par le fait que les juvéniles sont mobiles, très rapidement capables de se nourrir par eux-mêmes et donc qu'ils peuvent survivre en l'absence de leurs parents (Smiseth *et al.*, 2003; Costa, 2006; Kölliker, 2007). De par ces caractéristiques, et parce que la vie de famille est considérée comme une des premières étapes conduisant à l'évolution des formes complexes de vie sociale telle que l'eusocialité (Bourke & Franks, 1995; Bourke, 2011), le système précocial offre un modèle d'étude se rapprochant fortement du stade évolutif ayant vu le passage entre vie solitaire et vie de groupe (Smiseth *et al.*, 2003; Falk *et al.*, 2014) (Tableau 1).

Mes travaux de recherche essaient d'apporter une meilleure compréhension de l'évolution de la vie sociale chez les insectes en étudiant les mécanismes évolutifs limitant l'expression des conflits et/ou favorisant la coopération au sein de groupes sociaux présentant différents niveaux de complexité. Ma recherche s'est principalement appuyée sur deux espèces d'insectes aux systèmes sociaux radicalement différents. D'abord, la fourmi *Formica selysi* (recherches doctorales entre 2005 et 2009) avec sa vie sociale permanente et obligatoire (eusociale), qui m'a permis d'étudier l'importance de l'apparentement génétique sur la résolution des conflits dans les sociétés complexes d'insectes. Puis le forficule européen *Forficula auricularia* (depuis 2009) avec sa vie de famille précociale, qui m'a permis d'étudier les formes de coopérations et de conflits dans les structures sociales temporaires et facultatives. Dans l'ensemble, mes travaux utilisent une approche intégrative des différentes disciplines de l'écologie évolutive telles que l'écologie comportementale (quantification des comportements), l'écologie chimique (analyses des signatures chimiques des individus) et l'éco-immunologie (analyses spectrophotométriques de l'activité antimicrobienne), mais aussi des outils de la génétique quantitative (croisements contrôlés entre lignées) et des bio-statistiques (méta-analyses). Dans ce mémoire, j'ai choisi de présenter une partie de mes résultats à travers les deux grands axes que sont les conflits et la coopération. Cette organisation n'est pas chronologique et mélange une partie des travaux que j'ai réalisé lorsque j'étais doctorant (Lausanne, Suisse), post-doctorant (Bâle, Suisse), professeur assistant (Mayence, Allemagne) et chargé de recherche (Tours, France).

Guide de lecture

Les références à mes travaux sont indiquées en **gras** dans le texte et présentées dans l'encadré au début de chaque partie. Le nom des étudiants supervisés et associés à ces travaux est surligné dans ces références. Les astérisques (*) signalent les contributions à niveau égal entre les auteurs marqués.

2. LES CONFLITS SOCIAUX

Une grande partie des interactions ayant lieu au sein des groupes sociaux sont de nature conflictuelle (Krause & Ruxton, 2002a). Ces conflits peuvent être extrêmement coûteux pour les individus et leur résolution est donc considérée comme un élément central dans l'évolution de la vie de groupe (Ratnieks *et al.*, 2006). Depuis ma thèse de doctorat, mes travaux ont cherché à mieux comprendre la nature et l'importance de ces conflits au sein des sociétés complexes d'insectes telles que les colonies de fourmis et au sein des groupes sociaux structurés mais à l'organisation comparativement moins complexe telles que les unités familiales chez les forficules.

2.1 Dans les sociétés complexes

Chez les hyménoptères, le sexe des individus est déterminé par le niveau de ploïdie de chaque individu. Sauf cas exceptionnels (voir par exemple Pearcy *et al.*, 2004, 2011; Fournier *et al.*, 2005), les reines et les ouvrières des colonies de fourmis, guêpes et abeilles sont donc issues d'œufs fécondés et sont diploïdes, alors que les mâles sont issus d'œufs non-fécondés et sont haploïdes (Wilson, 1971). Ce système de détermination du sexe est connu pour entrainer des asymétries d'apparentement génétique entre les reines, les ouvrières et les mâles au sein d'une même colonie et donc pour générer des conflits d'intérêts entre ces castes (Trivers & Hare, 1976; Crozier & Pamilo, 1996; Burt & Trivers, 2006; Ratnieks *et al.*, 2006). Mais cette asymétrie de parenté peut aussi changer avec la structure sociale de la colonie, c'est-à-dire lorsque les colonies contiennent une ou plusieurs reines non apparentées (ou faiblement apparentées), lorsque la reine s'est accouplée avec un ou plusieurs mâles, ou encore lorsque la reine est remplacée par une de ses filles (Crozier & Pamilo, 1996). Ces variations de structures sociales sont donc supposées influencer l'intensité des conflits sociaux exprimés entre espèces, voire entre colonies d'une même espèce (Ratnieks *et al.*, 2006). Une partie de mes travaux de doctorat ont cherché à mieux comprendre la nature de l'association entre structure sociale et conflits sociaux chez les fourmis en se focalisant sur deux conflits : le conflit reine(s)-ouvrières sur la production de mâles et le conflit reine(s)-ouvrières sur le sex-ratio des descendants produits par les reines.

2.1.1 Conflit entre reines et ouvrières sur la production de mâles

- **Meunier J**, Delaplace L & Chapuisat M (2010). *Behav Ecol Soc*, 64(10), 1655–1663.

Même si elles ne s'accouplent pas, les ouvrières de nombreuses espèces de fourmis ont gardé des ovaires fonctionnels et ont donc la capacité de produire des œufs non-fécondés qui se transformeront en mâles (Wilson, 1971). Dans ces colonies, les ouvrières ont ainsi la possibilité de s'occuper du couvain mâle produit par leur reine (donc de leurs frères) et/ou du couvain mâle produit par les autres ouvrières (donc de leurs neveux). Parce que l'apparentement génétique des ouvrières envers chacun de ces types d'œufs change en fonction de la structure sociale de la colonie, il est attendu que cette structure sociale puisse influencer la préférence des ouvrières envers leurs frères ou leurs neveux. Ainsi, lorsque la colonie ne contient qu'une seule reine (colonie monogyne), les

ouvrières sont en moyenne plus apparentées à leurs neveux (r = 0.375) qu'à leurs frères (r = 0.25) et il est donc attendu que les ouvrières favorisent/tolèrent mieux les œufs produits par les autres ouvrières au sein de la colonie (Trivers & Hare, 1976; Crozier & Pamilo, 1996). Par contre, lorsque la colonie contient plusieurs reines (colonie polygyne avec des reines au moins partiellement apparentées – voir partie 2.1.2), ces niveaux moyens d'apparentement s'inversent et il est attendu que les ouvrières favorisent le couvain de la reine et donc empêchent la reproduction des autres ouvrières (Trivers & Hare, 1976; Crozier & Pamilo, 1996). Pour limiter cette reproduction, les ouvrières peuvent mettre en place des comportements d'agression envers les ouvrières fertiles et/ou éliminent directement les œufs produits par ces ouvrières, deux phénomènes regroupés sous le terme de *worker policing* (Ratnieks & Visscher, 1989; Monnin *et al.*, 2002; Ratnieks & Wenseleers, 2005). Il est important de noter que dans les ceux types de structure sociale, le maintien des œufs produits par les ouvrières va à l'encontre des intérêts génétiques de la reine – qui devrait monopoliser la production de mâles au sein de la colonie.

Nous avons testé l'effet de la structure sociale sur l'expression du *worker policing* chez la fourmi *Formica selysi* (Figure 3A). Dans cette espèce, les colonies peuvent contenir une ou plusieurs reines (jusqu'à 60 par nids). Ces deux formes sociales cohabitent au sein des mêmes populations (Purcell et al., 2015) et ne diffèrent pas en ce qui concerne le succès de fondation indépendante (c'est-à-dire que les nouvelles reines s'envolent pour fonder une nouvelle colonie seule) ou d'acceptation de nouvelles reines (**Reber *et al.*, 2010; Meunier *et al.*, 2011b**). Par contre, des différences ont été identifiées quant à leur durée de vie, leur nombre d'ouvrières, la taille des individus et l'odeur de leurs membres (Schwander *et al.*, 2005; **Meunier & Chapuisat, 2009**; **Meunier *et al.*, 2011a**). Malgré ces différences, un flux de gènes existe entre les deux formes sociales, démontrant qu'elles forment bien des populations

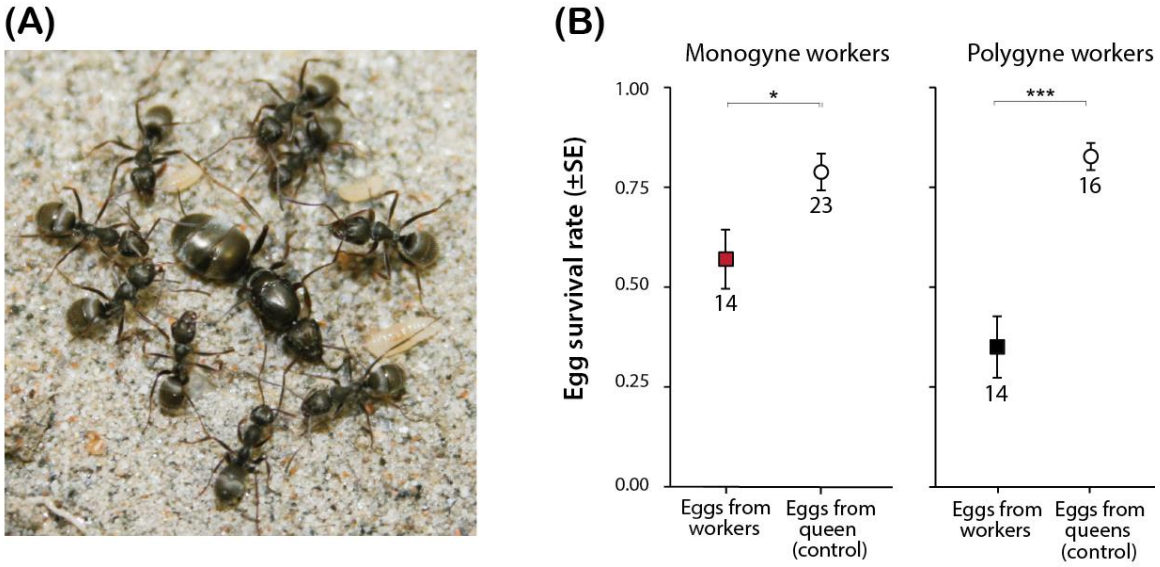

Figure 3 | (**A**) Ouvrières, reine et larves de la fourmi *F. selysi* (Photo : J. Meunier). (**B**) Les œufs produits par les ouvrières survivent moins que les œufs produits par les reines lorsqu'ils sont mis en présence d'ouvrières monogynes ou polygynes. Le niveau de cette discrimination n'est pas significativement différent entre les deux formes sociales. * P < 0.05. *** P < 0.001. Figure d'après Meunier *et al.* (2010).

génétiquement homogènes (Chapuisat *et al.*, 2004; Purcell & Chapuisat, 2013)(voir Purcell *et al.*, 2014 pour une étude récente des déterminants génétiques de ces structures sociales).

Afin de tester l'effet de la structure sociale sur l'expression du *worker policing*, nous avons proposé aux ouvrières (issues de colonies monogynes ou polygynes) des œufs produits par les ouvrières ou les reines de leur propre colonie. Au bout de 15 minutes, nous avons compté le nombre d'œufs récupérés par les ouvrières puis au bout de 24 heures, le nombre d'œufs qui n'avaient pas été détruits par les ouvrières (**Meunier *et al.*, 2010**). Nos résultats montrent que les œufs produits par les ouvrières sont récupérés moins rapidement et survivent moins bien que les œufs produits par les reines, quelle que soit l'origine sociale des ouvrières avec lesquelles ils ont été mis en présence (Figure 3B). Les ouvrières de *F. selysi* sont donc capables de reconnaitre et d'éliminer les œufs produits par les autres ouvrières, mais la structure sociale de la colonie n'a pas d'effet sur l'expression du *worker policing*. Ces résultats démontrent que le *worker policing* existe bien chez cette espèce de fourmis (tout comme dans de nombreuses autres espèces d'hyménoptères sociaux Ratnieks *et al.*, 2006), mais que ce phénomène n'est pas lié au conflit génétique entre la reine et les ouvrières sur la production de mâles dans la colonie. Comprendre l'émergence du *worker policing* reste un problème important dans la littérature et les tests expérimentaux cherchant à répondre à cette question restent souvent non-concluants (voir, par exemple, Gobin *et al.*, 2003; Ratnieks *et al.*, 2006; Dijkstra & Boomsma, 2008). A ce jour, l'hypothèse principale pour expliquer le *worker policing* est que la reproduction des ouvrières pourrait entrainer des coûts directs et indirects liés au fait d'avoir des ouvrières reproductrices dans la colonie, mais ces coûts qui restent à ce jour difficile à mettre en évidence (Olejarz *et al.*, 2016).

2.1.2 Conflit entre reines et ouvrières sur le sex-ratio de la colonie

- **Meunier J**, West SA & Chapuisat M (2008). *Behav Ecol*, *19*(2), 382–390

Dans les colonies d'hyménoptères eusociaux, un autre conflit important entre les reines et les ouvrières concerne le sex-ratio du couvain produit par les reines. Bien que ce conflit repose lui aussi sur le système haplo-diploïde de détermination du sexe, il concerne cette fois les différences d'apparentement génétique des ouvrières envers leurs frères et leurs sœurs. Dans les colonies monogynes et monoandres (la reine s'est accouplée avec un seul mâle), les ouvrières sont trois fois plus apparentées à leurs sœurs (r = 0.75) qu'à leurs frères (r = 0.25), si bien qu'elles devraient favoriser un sex-ratio trois fois plus biaisé vers les femelles que vers les mâles. Dans le même temps, les reines sont autant apparentées à leurs filles (r = 0.5) qu'à leurs fils (r = 0.5) et devraient donc favoriser un sex-ratio équilibré (Trivers & Hare, 1976; Crozier & Pamilo, 1996). Ces différences d'apparentement génétique entre reine et ouvrières constituent la base de leur conflit sur la proportion de femelles à produire au sein de la colonie. De façon intéressante, l'intensité de ce conflit peu aussi dépendre de la structure sociale. En effet, le remplacement de la reine par une reine fille, l'accouplement multiple des reines et la présence de plusieurs reines apparentées sont connus pour diminuer l'asymétrie de parenté des ouvrières envers les mâles et les femelles produits par la (les) reine, et devraient donc inhiber ce conflit (Ratnieks *et al.*, 2006).

Nous avons testé la force de ce lien théorique entre structure sociale et sex-ratio chez les hyménoptères sociaux au travers d'une méta-analyse (**Meunier *et al.*, 2008**). Pour cela, nous avons utilisé les résultats de 27 études conduites sur des espèces de fourmis, de guêpes et d'abeilles chez qui le sex-ratio des colonies a été corrélé aux variations d'asymétrie de parenté des ouvrières envers les deux types de sexués (variations dues à l'accouplement multiple des reines, à la présence de plusieurs reines apparentées ou au fait que la reine a été remplacée par une de ses filles). Nos résultats montrent que le sex-ratio de la colonie reflète principalement l'apparentement des ouvrières - plutôt que celui des reines - envers les deux types de sexués (Tableau 2). Cette étude révèle donc que lorsqu'il est présent, le conflit est généralement remporté par les ouvrières. Pour autant, nous montrons aussi que cette variation dans l'asymétrie de parenté n'explique que 20% des variations de sex-ratio entre les colonies, un chiffre qui est relativement élevé pour une méta-analyse, mais qui dénote l'importance de beaucoup d'autres facteurs sur ce sex-ratio d'une colonie. Chez certaines espèces, par exemple, d'élégantes études de croisement de couvain et de sexués ont démontré que les reines étaient capables de mettre en place des stratégies contre ce contrôle par les ouvrières, comme celle consistant à séparer dans le temps leur production de mâles et de femelles (Passera *et al.*, 2001; De Menten *et al.*, 2005; Rosset & Chapuisat, 2006). Les variations d'apparentement génétique entre les reines et les ouvrières semblent donc être un élément n'expliquant que partiellement le ratio entre les nouvelles reines et les mâles produits au sein des colonies d'hyménoptères sociaux.

Class of study Factor	Mean effect size (*r*)		Number of species
Relatedness asymmetry variation	0.457	**	7
Queen replacement	0.552	**	3
Mate number	0.368	*	4
Queen number variation	0.223	**	15
Monogyne versus polygyne colonies	0.09		9
Count of queens in polygyne colonies	0.24	**	4
From relatedness variation	0.354	**	6

Tableau 2 | *Effect size* moyen des études sur les ajustements de sex-ratio en fonction des variations d'apparentement, des variations du nombre de reines dans la colonie ou des interactions compétitives entre apparentés. * P < 0.05, ** P < 0.01. Tableau issu de Meunier *et al.* (2008).

2.2 Dans les unités familiales

Chez la majorité des espèces d'insectes, les adultes se rejoignent pour s'accoupler, puis les femelles choisissent un site de ponte dans lequel elles déposeront des œufs qui se développeront sans aide extérieure (Gullan & Cranston, 2005). Mais chez de nombreuses autres espèces, les parents restent avec les œufs jusqu'à l'éclosion et même parfois restent avec les juvéniles pendant plusieurs semaines (Trumbo, 2012; **Wong *et al.*, 2013**). Les interactions sociales ayant lieu au sein de ces unités familiales suivent généralement les

mêmes voies que celles présentes dans les colonies d'insectes eusociaux (fourmis, guêpes et abeilles) et donnent donc lieu à des conflits entre mère, père et juvéniles. Une partie de mes travaux sur le forficule européen (encadré 1) cherchent à améliorer notre compréhension générale des conflits (et de leur résolution) entre juvéniles, entre parents et enfants, et entre père et mère pendant la vie de famille.

2.2.1 Conflit entre juvéniles

- **Meunier J** & Kölliker M (2012). *Biology Letters*, *8*(4), 547–550.
- Wong JWY*, **Meunier J***, Lucas C & Kölliker M (2014). *Proceedings of the Royal Society B: Biological Sciences*, 281(1793), 20141236.

Au sein d'une famille, il est généralement attendu que chaque juvénile essaie de favoriser son propre développement en monopolisant l'accès aux ressources parentales telles que les soins ou la nourriture apportée par ces derniers (Mock & Parker, 1997). Ce conflit entre juvéniles est très présent dans la nature et de nombreuses études montrent qu'il engendre des coûts importants pour les juvéniles, notamment parce qu'il entraine l'expression de comportements agressifs aux effets parfois létaux pour les victimes (Roulin & Dreiss, 2012). Alors que l'apparentement génétique entre les membres d'une colonie est un facteur clé dans l'expression des conflits chez les insectes eusociaux (voir plus haut; Ratnieks *et al.*, 2006; **Meunier *et al.*, 2008**), l'effet de ce facteur sur les conflits entre juvéniles reste assez peu connu au sein des unités familiales simples (c.à.d. des systèmes subsociaux). Or, cet apparentement génétique varie chez un grand nombre d'espèces dans lesquelles les femelles s'accouplent avec plusieurs mâles. Au moment de notre étude, les seuls travaux explorant le rôle de la polyandrie sur le conflit entre juvéniles donnaient des effets contradictoires. D'un côté, il a été suggéré que la faible survie des juvéniles du Tétrix riverain *Tetrix subulata* (criquet) produits par les femelles polyandres était le résultat d'un plus grand conflit entre juvéniles (Caesar & Forsman, 2009), alors que d'un autre, l'effet de la polyandrie sur l'intensité du conflit entre juvéniles n'a pas été retrouvé ni chez le campagnol roussâtre *Myodes glareolus* (Klemme & Ala-Honkola, 2014) ni le guppy *Poecilia reticulata* (Evans & Kelley, 2008).

Nous avons testé le rôle de la polyandrie sur le conflit entre juvéniles chez le forficule européen *F. auricularia*. Chez cette espèce, nous avons montré que les femelles s'accouplent avec 1 à 4 mâles en milieux naturel et expérimental (Encadré 1 ; **Sandrin *et al.*, 2015**) et une autre étude révèle que les juvéniles se cannibalisent entre eux de manière assez fréquente lors de la vie de famille (Dobler & Kölliker, 2010). Dans notre expérience, nous avons accouplé les femelles avec 1 ou 4 mâles en laboratoire, puis manipulé le niveau de conflit entre juvéniles en mettant en place des groupes de 10 (faible conflit) ou 20 (fort conflit) juvéniles. Nous avons ensuite suivi le développement et la survie de ces juvéniles jusqu'à l'âge adulte (**Meunier & Kölliker, 2012b**). Nos résultats confirment que le conflit entre juvéniles est bien présent chez cette espèce. Il est plus intense dans les familles nombreuses, chez lesquelles il se matérialise par une diminution de la vitesse de développement des juvéniles et une diminution de la survie de ces derniers (Figure 4). Par contre, la polyandrie n'influence en rien

ces deux mesures (Figure 4). Contrairement au rôle supposé central de l'apparentement génétique entre les individus dans l'expression et la résolution des conflits sociaux (voir plus haut; Ratnieks *et al.*, 2006; **Meunier *et al.*, 2008**), nos résultats démontrent donc que cet apparentement ne détermine pas l'expression des conflits entre juvéniles chez le forficule.

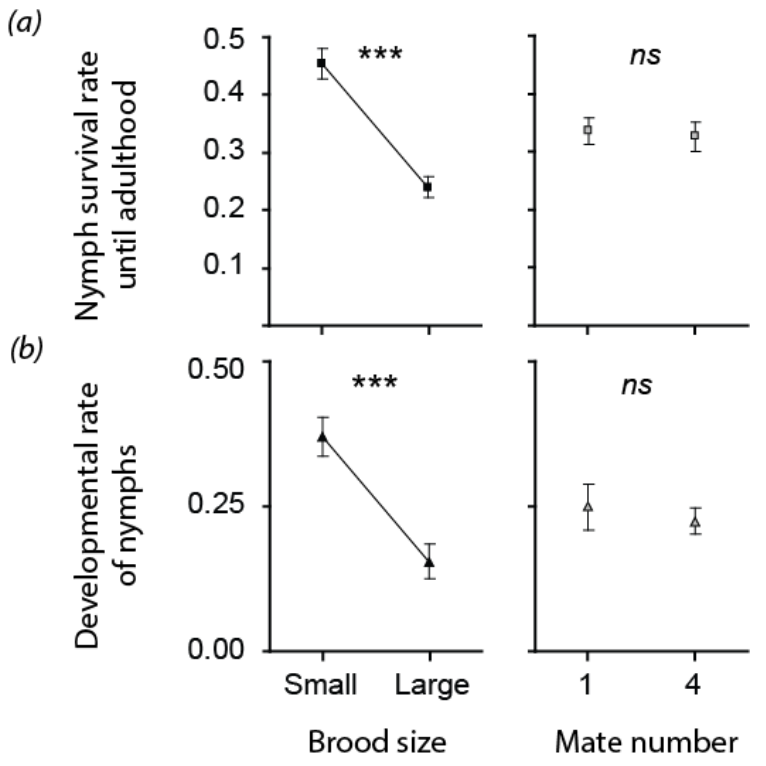

Figure 4 | Effets de la taille du groupe et de la polyandrie sur (**a**) la survie et (**b**) la vitesse de développement des juvéniles jusqu'à l'âge adulte. Le conflit entre juvéniles augmente avec la taille du groupe, mais est indépendant du nombre de lignées paternelles. *** P < 0.001. Figure d'après Meunier & Kölliker (2012b).

Il est toutefois important de souligner que ce que nous avons testé ne concerne que l'apparentement paternel. Or, chez les insectes eusociaux, plusieurs études suggèrent que le népotisme entre lignées paternelles est absent dans les colonies monogynes polyandres (Keller, 1997; Boomsma *et al.*, 2003; Holzer *et al.*, 2006)(mais voir Korb, 2006). Pour expliquer cette absence d'effet, il a été suggéré que le népotisme entrainerait des coûts trop importants pour les membres du groupe et que ce processus serait donc contre-sélectionné chez les espèces eusociales (Keller, 1997). Ces coûts pourraient être aussi présents dans les systèmes subsociaux, car les mères sont apparentées à tous leurs juvéniles de la même façon et devraient donc empêcher l'expression de comportements réduisant la survie d'une partie de leurs descendants. Pour autant, il reste une différence majeure entre les systèmes eusociaux et subsociaux : chez ces derniers, tous les descendants deviennent des reproducteurs. Dès lors, reconnaître les adultes apparentés des non-apparentés pourrait permettre d'éviter de s'accoupler avec des partenaires génétiquement proches – et ce même s'il s'agit uniquement de la lignée paternelle – et donc de limiter les risques de dépression de consanguinité. Chez les espèces subsociales, on pourrait donc s'attendre à ce que l'émergence de signaux permettant la reconnaissance des lignées paternelles soit contre-sélectionnée chez les juvéniles (pour empêcher le népotisme) mais sélectionnée chez les adultes (pour empêcher l'accouplement entre apparentés). Nous avons testé cette prédiction en étudiant les hydrocarbures cuticulaires (HC) des juvéniles du forficule européen. Chez les insectes, la reconnaissance des individus et la communication entre ces derniers dépendent en effet souvent des signaux chimiques présents sur leur cuticule (principalement des hydrocarbures ; Blomquist & Bagnères, 2010). Ces HC sont ainsi impliqués dans la reconnaissance du sexe des

individus qu'ils rencontrent, mais aussi de leur espèce, de leur nid d'origine, de leur apparentement génétique, de leur âge ou encore de leur activité (Blomquist & Bagnères, 2010; Wyatt, 2014; Leonhardt *et al.*, 2016). Chez le forficule européen, les HC présents sur les juvéniles et sur les mères sont connus pour refléter leur condition et modeler l'expression de certains comportements. Par exemple, une série d'études a montré que le niveau de soins

Encadré 1 - LE FORFICULE EUROPEEN

Le forficule européen *Forficula auricularia* - aussi appelé perce-oreille ou pince-oreille - est l'espèce de dermaptère la plus commune en Europe (Albouy & Caussanel, 1990; Gillott, 2005). Son cycle de vie est résumé dans la figure 5 et peut être entièrement reproduit en laboratoire **(Meunier & Kölliker, 2012a; b; Meunier *et al.*, 2012**). Le cycle de vie du forficule européen commence avec l'émergence des jeunes adultes au début du printemps (Figure 5a). Ces adultes sont grégaires (Raveh *et al.*, 2014; **Weiß *et al.*, 2014**) et s'accouplent avec plusieurs partenaires au cours de l'été (**Sandrin *et al.*, 2015**). A la fin de l'automne, les femelles s'isolent dans une cavité généralement creusée dans le sol pour y pondre 50 œufs en moyenne (**Meunier *et al.*, 2012**). Ces œufs sont gardés et régulièrement nettoyés par la femelle tout au long de l'hiver (Figure 5b), alors que les mâles sont maintenus éloignés des pontes qu'ils ont tendance à cannibaliser (**Boos *et al.*, 2014; Koch & Meunier, 2014**). Au début du printemps, les œufs éclosent en juvéniles (Figure 5c & 5d). Les mères restent avec ces juvéniles pendant plusieurs semaines, pendant lesquelles elles les protègent contre les prédateurs, les nettoient et leur apportent de la nourriture par régurgitation ou approvisionnement (Vancassel, 1984; Staerkle & Kölliker, 2008; **Meunier *et al.*, 2012**). La présence de la mère n'est pas requise pour assurer la survie des juvéniles, car ces derniers sont mobiles et peuvent fourrager dès l'éclosion des œufs (Kölliker, 2007; **Meunier & Kölliker, 2012a; b**). Pour autant, la mère reste avec ses juvéniles (et inversement) jusqu'à ce que ces derniers atteignent le second ou troisième stade de développement larvaire (ils en comptent quatre avant de devenir adultes). Les juvéniles quittent alors leur nid pour former de nouveaux groupes, qui peuvent inclure plusieurs dizaines d'individus des deux sexes (Moerkens *et al.*, 2009). Dans le même temps, certaines mères partent creuser un nouveau nid dans lequel elles produisent une deuxième ponte (**Meunier *et al.*, 2012**; Wong & Kölliker, 2014). Les juvéniles de cette deuxième ponte apparaissent au bout de deux semaines, puis deviennent des adultes avant l'été de la même année (Ratz *et al.*, 2016). Les mères mourront au cours de cet été, soit un peu plus d'une année après leur émergence.

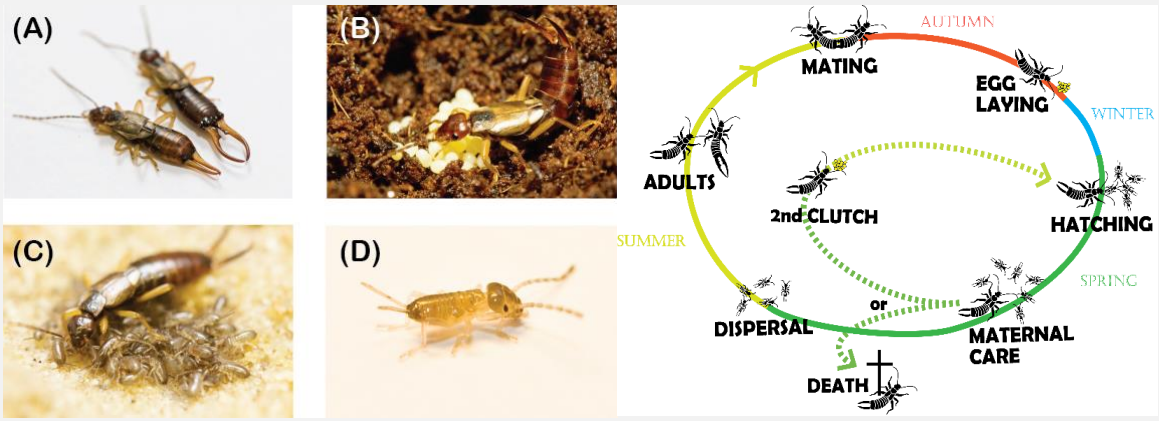

Figure 5 | Le forficule européen *Forficula auricularia*. (**A**) Une femelle et un mâle qui se distinguent uniquement à l'âge adulte par la forme droite des pinces de femelles et courbée de celle des mâles. **(**B) Une femelle s'occupant de ses œufs. (**C**) Une femelle s'occupant de ses juvéniles âgés de quelques jours. (**D**) Un juvénile âgé de sept jours. (**E**) Le cycle de vie du forficule européen, ce cycle dure environ 18 mois. (Crédits photos : J. Meunier)

maternels change lorsque les femelles sont exposées à des HC extraits de juvéniles sous- ou bien-alimentés (Mas *et al.*, 2009), que cette exposition détermine le délai observé par les mères avant de produire une deuxième ponte (Mas & Kölliker, 2011), mais aussi que l'exposition des juvéniles à des extraits d'HC de mères sous- ou bien-alimentées influence leur niveau de cannibalisme (Wong *et al.*, 2014a).

Nous nous sommes donc intéressés au rôle des pères et de l'âge des descendants dans la composition des signatures chimiques de ces derniers chez le forficule européen (**Wong *et al.*, 2014b**). Plus spécifiquement, nous avons d'abord expérimentalement accouplé des femelles avec 1 ou 4 mâles, puis nous avons extrait les HC de descendants lorsque ces derniers étaient des juvéniles (c.à.d. pendant la vie de famille) puis des adultes. Nous avons ensuite testé 1) s'il y avait une signature chimique propre à chaque famille et 2) si cette signature était altérée chez les familles polyandres (du fait des signaux propres aux pères) et enfin 3) si cette altération était plus forte chez les adultes que chez les juvéniles. Nos résultats sont allés dans le sens de nos prédictions (Figure 6). Nous avons trouvé que la signature chimique d'un individu permettait de l'assigner correctement à sa famille d'origine et que ce succès d'assignement était altéré lorsque les descendants adultes – mais pas juvéniles - étaient issus de familles polyandres. Dans l'ensemble, nos résultats suggèrent donc que l'absence de népotisme entre juvéniles démontrée précédemment (**Meunier & Kölliker, 2012b**) pourrait reposer sur l'absence de signaux (chimiques) permettant de reconnaître les lignées

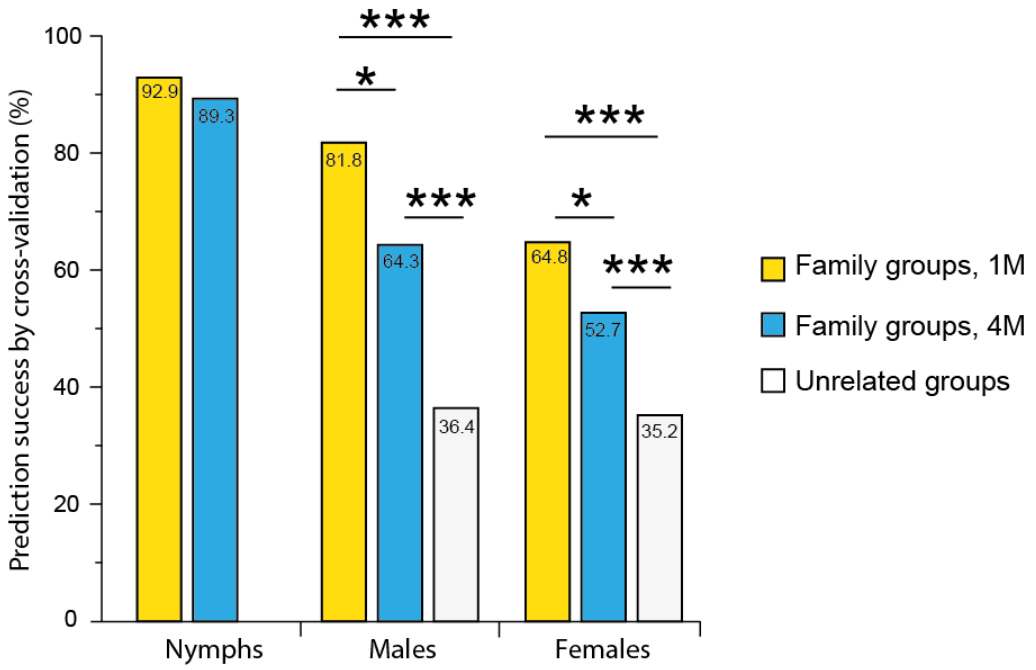

Figure 6 | Effet de la polyandrie sur le succès de discrimination de l'origine des juvéniles (nymphs) et des nouveaux adultes (males et females) basée sur leur signature chimique. Les mères de chaque groupe étaient accouplées avec un seul mâle (1M) ou quatre mâles (4M). Des groupes constitués d'individus non-familiers ont été mis en place en guise de contrôle (Unrelated groups). Les résultats ont été obtenus avec des analyses discriminantes. Dans l'ensemble, la signature chimique des individus est plus caractéristique de leur famille d'origine dans les familles monoandres que dans les familles polyandres, mais cette différence n'est vraie que lorsque les descendants sont devenus des adultes. * $P < 0.05$, *** $P < 0.001$. Figure d'après Wong *et al.* (2014b).

paternelles au sein du groupe familial. Cette absence pourrait être due soit aux soins maternels - qui seraient utilisés pour homogénéiser les signatures chimiques de ses juvéniles et ainsi prévenir les coûts du népotisme, soit à des contraintes propres aux juvéniles (par exemple physiologiques) qui les empêcheraient de synthétiser les HC spécifiquement impliqués dans la signature paternelle. Quel que soit le mécanisme responsable de ces résultats, nos résultats suggèrent que le rôle de l'apparentement génétique dans l'expression des conflits en juvéniles est potentiellement présent chez un grand nombre de formes sociales, mais qu'il n'implique pas forcément le matériel génétique donné par les pères.

2.2.2 Conflit entre parents et enfants

- Kölliker M, Boos S, Wong JWY, Röllin L, Stucki D, Raveh S, … & **Meunier J** (2015). *Nature Communications*, 6, 6850.

Le second type de conflit est celui qui oppose les parents et les juvéniles sur l'investissement des premiers dans les soins parentaux (Kilner & Hinde, 2012). Ce conflit, qui est une pierre angulaire de la sélection de parentèle et de la vision gène-centré de l'évolution, est basé sur le fait que les juvéniles sont sélectionnés pour demander plus de soins (dus aux bénéfices associés, par ex. en matière de survie) que les parents sont sélectionnés à donner (dus à leurs coûts, par exemple en matière de reproduction future). Depuis que ce conflit a été formulé (Trivers, 1974), beaucoup d'études comportementales se sont intéressées à son existence en analysant qui avait le contrôle du niveau de soin parental (Royle *et al.*, 2002; Kilner & Hinde, 2008). Mais les résultats de ces études sont considérés comme des preuves indirectes de l'existence de ce conflit et donc de son implication dans l'évolution de la vie de famille. En effet, ce conflit présuppose l'existence d'un compromis génétique entre l'investissement des parents dans la reproduction future et la performance des enfants (Trivers, 1974; Lundberg & Smith, 1994; Kölliker *et al.*, 2010), un processus qui au moment de notre étude n'avait jamais été démontré. C'est ce que nous avons fait en mettant en place des lignées de sélection chez le forficule européen (Kölliker *et al.*, 2015). Pendant six générations (soit une expérience de presque cinq ans), nous avons sélectionné les mères sur leur niveau d'investissement dans la reproduction future (i.e. petit ou grand investissement dans une deuxième et dernière ponte) et mesuré les réponses à cette sélection chez les mères et leurs juvéniles. Comme prédit, nos résultats montrent une réponse directe des mères à la sélection, avec un investissement dans la taille de la deuxième ponte qui augmente ou diminue en fonction du type de sélection (Figure 7 ; Kölliker *et al.*, 2015). Mais plus important, nos résultats démontrent aussi que la sélection sur les mères entraine des réponses indirectes et antagonistes dans la performance des juvéniles. Par exemple, les juvéniles produits dans les lignées où les femelles investissaient beaucoup dans leur deuxième ponte avaient une vitesse de développement, une survie et une prise de poids plus faibles que dans les lignées où les femelles investissaient moins dans leur deuxième ponte (Figure 7). Nos résultats démontrent donc de façon empirique qu'investir dans la reproduction future en tant que femelle adulte se traduit par une qualité amoindrie des juvéniles (et inversement). Avec ces résultats, notre étude a donc apporté la première preuve directe de l'existence de bases génétiques au conflit entre parents et enfants formulé par Trivers dès 1974.

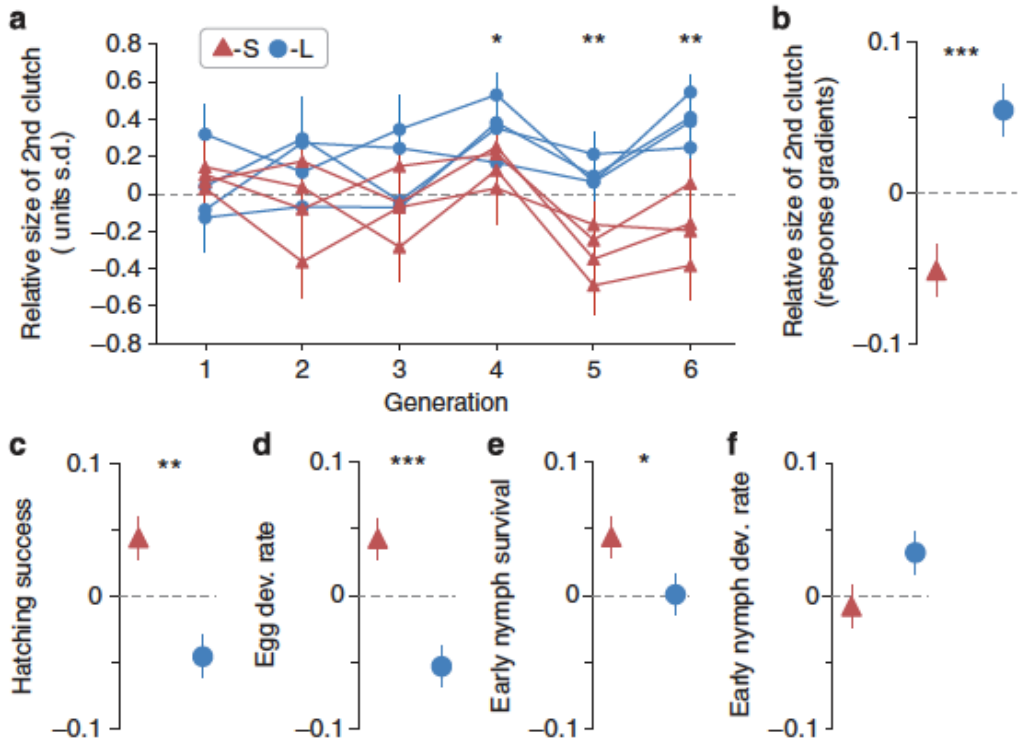

Figure 7 | Réponses directes et indirectes à deux sortes de sélection. La réponse directe (**a, b**) se mesure au travers de l'évolution de la moyenne de la taille relative de la deuxième ponte produit par les femelles par rapport à la taille de leur première ponte. Les réponses indirectes sont représentées pour la dernière génération et reflète (**c**) le taux d'éclosion, (**d**) la vitesse de développement des œufs, (**e**) le taux de survie des juvéniles et (**f**) la vitesse de développement des juvéniles. Les valeurs correspondent aux différences entre les valeurs des lignées de sélection et celles des lignées contrôles. S = sélection des femelles avec un faible investissement dans la reproduction future. H = sélection des femelles avec un fort investissement dans la reproduction future. D'après Kölliker *et al.* (2015).

2.2.3 Conflit entre le(s) père(s) et la mère

- **Meunier J** & Kölliker M (2012). *Proceedings of the Royal Society B: Biological Sciences*, 279(1744), 3981–8.

Le troisième conflit qui peut s'exprimer au sein d'une famille est celui opposant les deux parents sur leur investissement respectif dans les soins aux juvéniles (Lessells, 2012). Il est important de noter que ce conflit n'est pas uniquement présent dans les espèces où les deux parents s'occupent des juvéniles après leur émergence, mais qu'il est aussi présent dans celles où un seul des parents est impliqué dans ces soins. C'est le cas, notamment, dans les familles où les femelles s'accouplent avec plusieurs mâles et s'occupent seules des juvéniles. La « kinship theory of genomic imprinting » (Haig, 2000, 2004) prédit ainsi que les gènes hérités par les pères et exprimés chez les juvéniles devraient être sélectionnés s'ils permettent d'exagérer le niveau de demande de soins maternels par les juvéniles car ils permettraient aux mâles de favoriser leurs propres descendants face à ceux des autres mâles au sein d'une même couvée. Dans le même temps, cette théorie prédit que les gènes hérités par les mères et exprimés chez les juvéniles devraient être sélectionnés s'ils permettent de limiter le niveau de demande de soins maternels car ils pourraient à la fois favoriser la survie de tous les descendants de la

femelle et permettre à cette dernière de ne pas surinvestir dans la couvée présente afin de pouvoir le faire pour une couvée future (Haig, 1997, 2000). Cet antagonisme parental sur l'expression des gènes (qui peut prendre forme au travers d'une empreinte génomique) a été montré dans plusieurs espèces de vertébrés (Itier *et al.*, 1998; Lefebvre *et al.*, 1998; Hager & Johnstone, 2003; Curley *et al.*, 2004), chez qui le dérèglement de son expression est associé à des pathologies sérieuses dans le cadre du développement embryonnaire (Burt & Trivers, 2006). Mais un rôle potentiellement important et encore peu considéré (voire totalement ignoré) au moment de notre étude est celui qu'il pourrait jouer dans le fonctionnement des mécanismes évolutifs impliqués dans la résolution du conflit parent-enfant. Le conflit parent-enfant peut être résolu au travers d'un phénomène de coadaptation consistant à sélectionner au sein d'un individu une combinaison de stratégies parentales et juvéniles maximisant la fitness de cet individu pendant les deux étapes de son développement (Kölliker *et al.*, 2005; Hinde *et al.*, 2010). En d'autre terme, une stratégie trop bénéfique en tant que juvénile (c'est-à-dire une stratégie visant à manipuler efficacement sa mère et donc obtenir plus de soins parentaux au détriment de sa propre mère) serait ensuite couteuse en tant qu'adulte, car elle serait transmise à ses descendants et se retournerait alors contre le porteur de cette stratégie devenu adulte (Kölliker *et al.*, 2012). Ce phénomène demande donc que les individus soient à la fois des receveurs (en tant que juvéniles) et des donneurs (en tant qu'adulte prodiguant des soins à ses juvéniles) au cours de leur vie. Cette condition n'étant pas remplie par les lignées paternelles dans les familles où seule la mère prodigue des soins, il est attendu que la coadaptation (sur les lignées maternelles) et l'antagonisme parental aient des effets opposés sur les gènes responsables de l'expression (et de la réception) de ces soins. Malgré des bases théoriques solides et des conséquences potentiellement majeures sur l'évolution de la vie de famille (et la résolution des conflits au sein de ces familles), les effets de l'antagonisme parental sur le phénomène de coadaptation n'avaient - au moment de notre étude - jamais été exploré de façon expérimentale.

Nous avons donc testé expérimentalement si l'antagonisme parental peut agir sur le phénomène de coadaptation chez le forficule européen. Afin de démêler les effets de ces deux processus, il est important de pouvoir manipuler les sources potentielles de conflits en créant des familles où la mère, le père et les juvéniles expriment des stratégies différentes (et

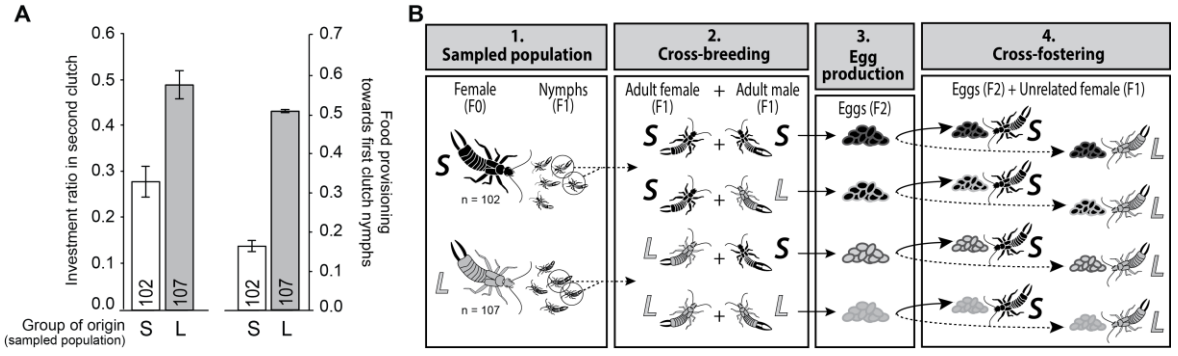

Figure 8 | Design expérimental permettant de démêler le rôle de l'origine des mères biologiques, des mères adoptives et des pères biologiques sur l'approvisionnement en nourriture. Les individus ont été produits dans des familles avec un haut (L pour Large) ou faible (S pour Small) niveau d'approvisionnement en nourriture des juvéniles – voir partie de gauche. Tiré de Meunier & Kölliker (2012a).

connues) en ce qui concerne les soins parentaux. Nous avons donc fait ce type de manipulation en réalisant des accouplements contrôlés suivis d'adoptions croisées d'œufs **(Meunier & Kölliker, 2012a)**. Ces croisements impliquaient des mâles et des femelles dont les mères avaient beaucoup (L pour « large ») ou peu (S pour « small ») investi dans la taille de sa deuxième couvée et dans l'apport en nourriture de leurs juvéniles (ces deux traits sont confondus, voir Meunier *et al.*, 2012). Au total, 8 types de croisements ont été réalisés de sorte à avoir des familles expérimentales chez qui les membres avaient toutes les combinaisons possibles d'origines L ou S entre la mère adoptante (donc qui prodigue les soins mesurés, OFM pour « Origin of Foster Mother »), la mère biologique des juvéniles (OGM pour « Origin of Genetic Mother ») et le père biologique des juvéniles (OGF pour « Origin of Genetic Father ») (Figure 8). Si un antagonisme parental était responsable des soins aux œufs, nous prédisions que le niveau de ces soins dans les familles expérimentales serait déterminé soit par le père (OGF) ou la mère (OGM) des juvéniles, soit par une interaction entre ces deux facteurs. Dans le même temps, si une coadaptation avait lieu, nous prédisions que le soin aux juvéniles serait déterminé par une interaction entre la mère adoptante (OFM) et la mère biologique (OGM), de sorte qu'une similarité entre ces deux origines devrait favoriser l'expression de ces soins. Nos résultats suivent donc non seulement ces deux prédictions, mais révèlent aussi que ces deux phénomènes sont liés (Figure 9). En particulier, les soins aux

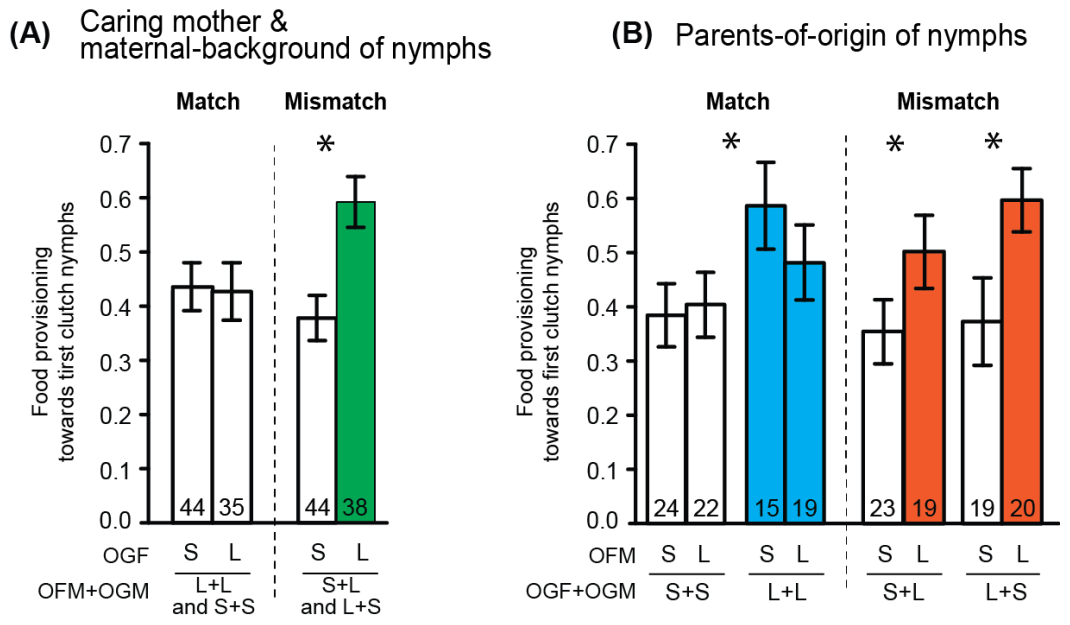

Figure 9 | Co-adaptation et antagonisme parental interagissent pour déterminer le niveau d'approvisionenement de nourriture par les mères. OGF = Origine des mères biologiques (Origin of Genetic Mother). OFM = origine des mères adoptantes (Origin of the Foster Mother). OGM = Origine des pères biologiques (Origin of the Genetic Father). Les origines indiquent que les individus ont été produits par des femelles ayant investi beaucoup (L pour Large) ou peu (S pour Small) dans leur investissement dans une ponte future et dans l'apport de nourriture aux juvéniles. (**A**) L'origine génétique de la mère des juvéniles détermine le niveau de nourriture apporté par la mère adoptante, mais uniquement lorsque les origines de la mère adoptante et du père génétique sont différents. (**B**) Lorsque les deux parents génétiques du juvénile ont la même origine, alors leur origine détermine le niveau de nourriture apporté par la femelle adoptante. Par contre, lorsque ces deux origines diffèrent, c'est l'origine de la femelle adoptante qui détermine ce niveau. Figure adaptée de Meunier & Kölliker (2012a).

juvéniles sont déterminés par leur père biologique uniquement lorsque la mère biologique et la mère adoptante ont une origine différente (Figure 9a). De même, l'origine de la mère adoptante détermine le niveau de soins uniquement lorsque le père et la mère biologique ont des origines différentes (Figure 9b). Ces résultats démontrent donc que l'antagonisme parental et la coadaptation parent-enfant sont deux processus présents et dont les effets sur les soins aux juvéniles sont inter-dépendants chez les forficules. Il s'agit de la première démonstration d'une forme d'antagonisme parental (et potentiellement d'empreinte génomique) chez un invertébré, indiquant que ce mécanisme ne nécessite pas d'échanges placentaires pour émerger – une condition souvent mise en avant – et donc qu'il pourrait être très présent dans la nature (Itier *et al.*, 1998; Lefebvre *et al.*, 1998; Hager & Johnstone, 2003; Curley *et al.*, 2004). De plus, notre étude met en avant l'importance de considérer ces deux processus évolutifs majeurs ensemble plutôt que séparément si l'on veut appréhender les mécanismes régulant les conflits familiaux et favorisant l'évolution de la vie sociale. Elle démontre aussi que l'environnement social expérimenté lors du jeune âge d'un individu détermine la stratégie qu'il va plus tard adopter en tant que parent mais aussi qu'il va transmettre à ses propres descendants. En conséquence, la nature des interactions parents-enfants peut être à la fois la cause et la conséquence de la variation héritable des stratégies des membres de la famille, ce qui donne un exemple frappant de l'importance de ces effets réciproques en biologie évolutive (Laland *et al.*, 2011).

3. LA COOPÉRATION

Le moteur principal de l'évolution de la vie sociale est le fait que les individus ont trouvé des bénéfices nets à la vie de groupe (Bourke, 2011). Ces bénéfices sont principalement associés à la coopération (Krause & Ruxton, 2002a), un phénomène qui peut prendre de nombreuses formes et impliquer différents partenaires au sein d'un groupe. L'autre axe principal de mes recherches se focalise donc sur l'importance et la nature de la coopération dans l'évolution de la vie sociale. Je me suis particulièrement intéressé à la coopération dans les unités familiales et donc aux soins parentaux, aux interactions (non-compétitives) entre juvéniles et à la protection collective contre les pathogènes et les parasites.

3.1 Les soins parentaux

- Wong JWY, **Meunier J** & Kölliker M (2013). *Ecological Entomology*, 38(2), 123–137.

Dans le cadre de la vie de famille, une forme claire et bien étudiée de coopération concerne les soins des parents envers les juvéniles. L'expression de ces soins est un phénomène très commun chez les vertébrés (tels que les oiseaux et les mammifères), mais comparativement beaucoup moins fréquent chez les insectes et les arachnides (Choe & Crespi, 1997). Dans la nature, ces soins peuvent être prodigués par un ou deux parents, durer de quelques jours à plusieurs semaines et prendre des formes variées allant de la protection contre les prédateurs à l'approvisionnement en nourriture (Smiseth *et al.*, 2012; Klug & Bonsall, 2014). Parce que ces soins peuvent être coûteux pour les parents,

un problème majeur en biologie évolutive est de comprendre les conditions ayant permis leur émergence et leur maintien au cours du temps. C'est la question principale à laquelle nous nous sommes intéressés dans une review publiée en 2013 et portant sur les soins parentaux chez les insectes **(Wong *et al.*, 2013**). En particulier, nous avons cherché dans la littérature des études nous permettant de savoir si – comme il est traditionnellement prédit (Wilson, 1975) - les bénéfices associés aux soins parentaux étaient particulièrement importants lorsque (1) les conditions environnementales sont difficiles, (2) les ressources alimentaires sont éphémères, distantes du nid et/ou les individus ont un régime nutritionnel spécialisé, (3) la pression exercée par les ennemis naturels est forte et (4) l'environnement abiotique est prévisible. Nos résultats révèlent que les preuves empiriques démontrant l'importance des deux premiers points sont contrastées et donc que le rôle de ces deux paramètres dans l'évolution des soins parentaux pourrait dépendre principalement de l'espèce concernée. Par contre, les études s'intéressant à l'importance des ennemis naturels et de la stabilité du milieu sur les soins parentaux vont dans le même sens et démontrent l'importance de ces paramètres. Il semblerait donc que ces quatre paramètres n'aient pas le même effet intrinsèque sur les bénéfices des soins parentaux, et ainsi qu'ils ne soient pas nécessairement des facteurs universels favorisant l'apparition et le maintien des soins parentaux chez les insectes. En plus de ces facteurs, nous mettons aussi en avant l'importance de l'environnement social sur l'émergence et le maintien des soins parentaux – un paramètre souvent négligé dans la littérature sur l'évolution des soins parentaux. En effet, cet environnement social (c'est à dire les autres membres de la famille) n'exerce pas uniquement une pression de sélection sur un individu receveur de soins, mais il est lui-même modelé en retour par la pression de sélection exercée par les individus présents qui peuvent être des donneurs et des receveurs. Etudier l'évolution des soins parentaux, c'est donc aussi s'intéresser aux boucles de rétroactions entre donneurs et receveurs et aux pressions de sélection agissant à la fois sur une seule et sur les deux parties simultanément. Comprendre ce système complexe de rétroaction est donc crucial pour élucider le déterminisme des soins parentaux (par exemple les phénomènes de coadaptations ; Smiseth *et al.*, 2008) et étudier son histoire évolutive au travers des espèces.

3.1.1 Investir ou non dans les soins aux œufs ?

- Koch LK **& Meunier J (2014).** *BMC Evolutionary Biology, 14(1), 125.*

Les femelles ont généralement deux façons de maximiser leur production de descendants : elles peuvent investir dans les soins qu'elles prodiguent à leurs œufs (investissement post-ponte) et dans le nombre et/ou la qualité de leurs œufs (investissement pré-ponte) (Smiseth *et al.*, 2012). Alors que de nombreuses études ont révélé qu'un compromis entre quantité et qualité des œufs existe chez la plupart des vertébrés et des invertébrés (Shine, 1989; Krist, 2011), la présence et la nature du lien entre ces investissements et l'expression de soin maternels étaient peu connues au moment de notre étude (Summers *et al.*, 2006). Etudier ce lien est pourtant primordial si l'on veut mieux comprendre le rôle des soins parentaux dans l'évolution des stratégies de reproduction (Stearns, 1992) et notamment déterminer si investir dans les soins maternels post-éclosion est une stratégie fixe et prédéterminée, ou un processus plastique visant à compenser une

capacité d'investissement limité dans la quantité et/ou la qualité des œufs produits (Lock *et al.*, 2007).

Nous avons testé si la quantité et la qualité des juvéniles produits dépendaient de l'investissement des mères dans la quantité et la qualité des œufs produits, ainsi que dans les soins maternels envers les œufs (**Koch & Meunier, 2014**). En particulier, nous avons utilisé 80 femelles sur lesquelles nous avons mesuré le nombre d'œufs produits, le poids moyen de ces œufs, la perte de poids des mères entre la production des œufs et leur éclosion (une mesure reflétant l'investissement dans les soins maternels, car les femelles ne se nourrissent pas pendant la période de soins aux œufs (Kölliker, 2007)), le nombre de juvéniles produits et leur poids moyen. Nos résultats montrent dans l'ensemble que la qualité des juvéniles est uniquement déterminée (positivement) par le poids des œufs, alors que les trois types d'investissements maternels interagissent pour déterminer le nombre de juvéniles produits. Ces interactions montrent d'abord que l'association positive entre nombre d'œufs et nombre de juvéniles disparait lorsque les œufs sont de faible poids (Figure 10A) ou lorsque les femelles investissent peu dans les soins aux œufs (Figure 10B). Ces résultats révèlent donc à la fois que la qualité intrinsèque des œufs dépend de leur poids, et que l'investissement des femelles dans les soins aux œufs peut favoriser la capacité des œufs à se développer jusqu'à l'éclosion. Ensuite, nos résultats montrent que le poids des œufs est associé positivement au nombre de juvénile produits lorsque les femelles investissent dans le soin aux œufs, mais que cette association devient négative lorsque cet investissement se réduit (Figures 10C). Ainsi, les œufs les plus gros semblent avoir un besoin plus important de soins maternels que les œufs les plus petits, mais les œufs les plus gros permettent aussi d'avoir les juvéniles de meilleure qualité. Ce résultat révèle donc qu'investir dans les soins maternels après la production des œufs pourrait être une stratégie des femelles visant à compenser un investissement réduit dans la qualité des œufs. Dans leur ensemble, ces résultats démontrent qu'un lien complexe entre les investissements maternels exprimés avant ou après la production des œufs détermine le succès reproducteur des femelles. De manière général, cette étude met aussi en avant l'importance de prendre en compte les compromis entre traits d'histoire de vie si l'on veut comprendre les facteurs responsables de la fitness des individus (Stearns, 1992; Flatt & Heyland, 2011).

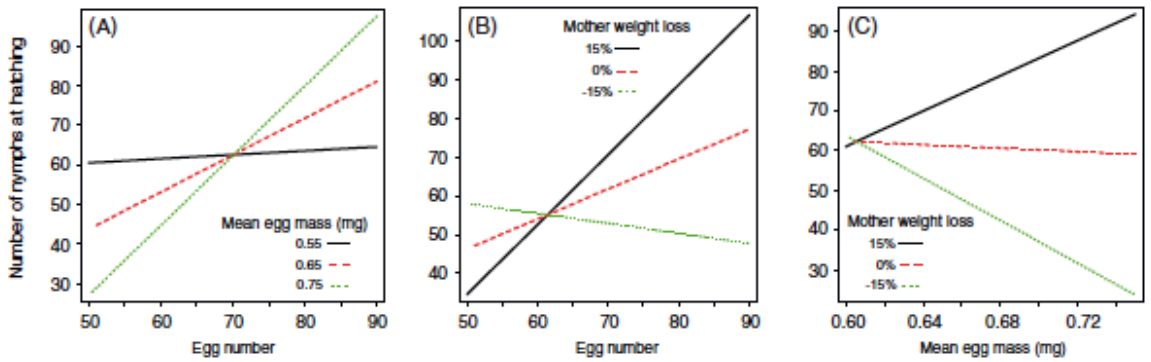

Figure 10 | Effets des interactions entre le nombre d'œufs, le poids des œufs et l'investissement dans le soin aux œufs sur le nombre de juvéniles produits. Les droites représentent les corrélations prédites par les modèles statistiques (GLM) pour des valeurs théoriques données et correspondant au 1er quartile, à la médiane et au 3ème quartile de la distribution de ces données. Figures tirée de Koch & Meunier (2014).

3.1.2 Des bénéfices pour les juvéniles sur le court et le long terme ?

- Thesing J, Kramer J, Koch LK & **Meunier J** (2015). *Proceedings of the Royal Society B: Biological Sciences*, 282(1817), 20151617.

La présence des parents avec les juvéniles est souvent associée à l'expression de soins parentaux et donc à des bénéfices pour les descendants (Smiseth *et al.*, 2012; Klug & Bonsall, 2014). De ce fait, l'absence ou la perte des parents sont souvent vues comme une source de coûts majeurs pour les juvéniles et ces coûts comme un moteur dans le maintien de la vie de famille d'une génération à l'autre. Alors que les coûts liés à l'absence ou la perte des parents sont généralement directs et influencent le développement, la survie ou le succès reproducteur des juvéniles une fois devenus adultes (Harlow & Suomi, 1971; Foster *et al.*, 2012; Andres *et al.*, 2013), ces coûts peuvent être aussi transgénérationnels et par exemple affecter l'expression des soins parentaux chez la génération suivante (Gonzalez *et al.*, 2001). Pour autant, l'existence de coûts transgénérationels est uniquement connue chez des espèces altriciales, où la survie des juvéniles dépend fortement de la présence des parents. Démontrer leur existence dans les espèces précociales est pourtant très important, car les coûts transgénérationnels pourraient y favoriser le maintien de la vie de famille lorsque la perte des parents a un coût direct limité (voire absent) pour les juvéniles – un scénario qui reflète les premières étapes de l'évolution de la vie de groupe (West *et al.*, 2015). Nous avons donc testé les effets à court-terme, long-terme et transgénérationnels de la perte de la mère chez le forficule européen (**Thesing *et al.*, 2015**). Pour ce faire, nous avons élevé des juvéniles avec ou sans leur mère jusqu'à l'âge adulte, fait des accouplements croisés entre les deux catégories de nouveau adultes produits et enfin fait un échange d'œufs entre les mères

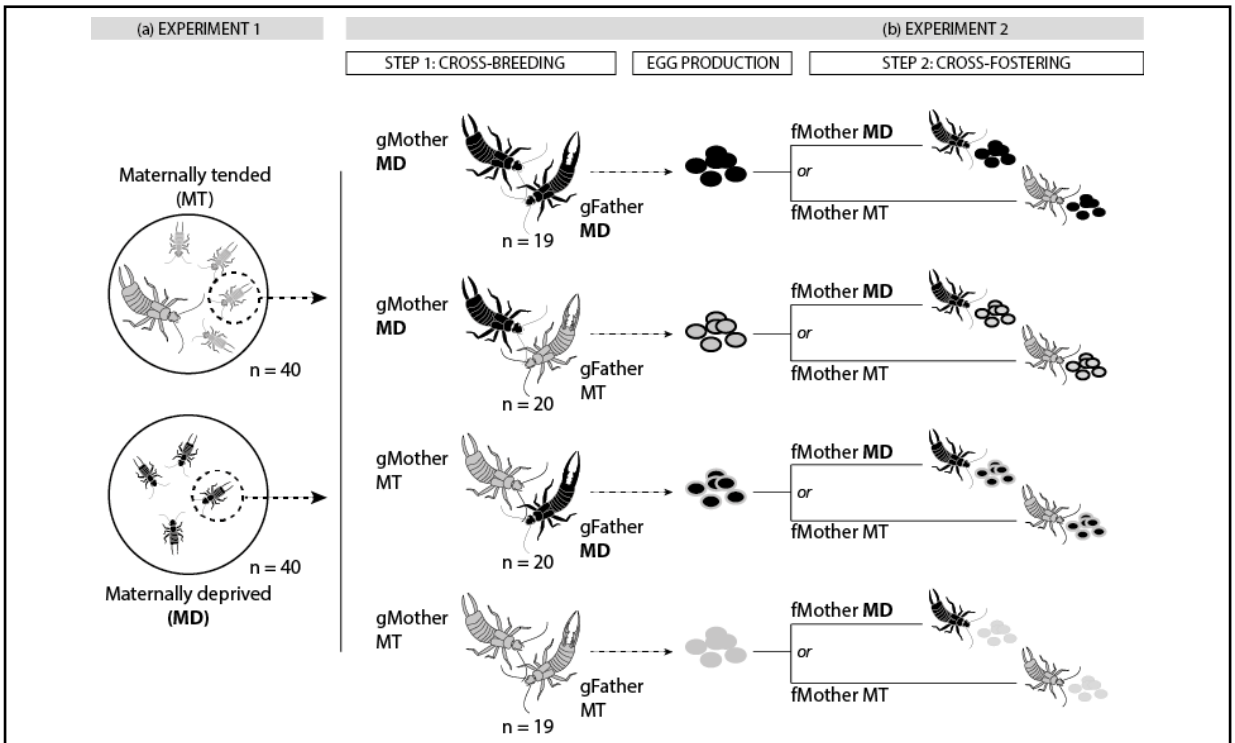

Figure 11 | Design expérimental permettant de démêler le rôle de l'origine des mères biologiques (gMother), des mères adoptives (fMother) et des pères biologiques (gFather) sur les soins maternels et divers traits d'histoire de vie à la génération suivante. Adapté de Thesing *et al.*, (2015).

résultantes de ces accouplements (Figure 11). Un tel processus nous a permis d'obtenir des familles expérimentales dans lesquelles nous pouvions tester si l'expression des traits d'histoires de vie (dont les soins maternels) était déterminée par le passé de la mère adoptive (orpheline ou non), le passé de la mère qui a pondu les œufs (orpheline ou non), le passé du père qui a fertilisé les œufs (orphelin ou non) ou par une interaction entre ces trois facteurs.

Nos résultats montrent d'abord que la présence de la mère n'améliore pas le taux de survie des juvéniles jusqu'à l'âge adulte (avant nos croisements expérimentaux, donc) (Thesing *et al.*, 2015). Ce résultat confirme celui d'une étude précédente (Kölliker, 2007) et démontre bien que les soins maternels sont facultatifs chez cette espèce. Par contre, nous montrons ici que la présence de la mère présente un coût pour les descendants car elle induit la production d'adultes plus petits et avec des pinces moins longues - deux propriétés connues pour réduire la fitness des forficules (Tomkins & Simmons, 1998; Rantala *et al.*, 2007). A première vue, ce coût net pour les juvéniles est particulièrement surprenant, car il suggère que la vie de famille ne devrait pas être maintenue dans la nature - alors qu'elle y est bien présente. Une explication à ce résultat surprenant est que ces coûts ont été découverts dans des conditions expérimentales standardisées excluant *de facto* des pressions de sélection qui pourraient être importantes comme, par exemple, la présence de pathogènes et/ou de prédateurs à proximité du nid. Il est donc fort probable que la présence de la mère apporte bien des bénéfices nets à ses juvéniles en conditions naturelles, mais simplement que nos conditions expérimentales ne permettent pas à ces bénéfices de s'exprimer. Cela étant dit, nos conditions expérimentales ont permis de mettre en lumière la présence de coûts jusqu'ici peu connus pour les juvéniles et qui sont associés à la présence de la mère. Nos résultats suggèrent donc que ces coûts devraient être pris en compte lorsque l'on s'intéresse à la somme des processus capables de favoriser ou d'inhiber l'évolution de la vie de famille à partir d'un état solitaire.

Les résultats de notre deuxième étape expérimentale, c'est-à-dire les croisements, démontrent quant à eux que la perte de la mère réduit l'expression des soins maternels par les nouveaux adultes. Ce coût transgénérationnel n'est pas seulement dicté par le passé des mères adoptives, mais aussi par le passé des parents biologiques des juvéniles (**Thesing *et al.*, 2015**). En particulier, nous démontrons que les mères adoptives qui ont été orphelines procurent moins de soins aux juvéniles que les mères adoptives qui ont été élevées par leur mère (Figure 12a et b). Indépendamment de cet effet, nous montrons aussi que les juvéniles produits par les femelles orphelines sont mieux défendus par les mères adoptantes que les juvéniles produits par les femelles ayant été élevées avec leur mère (Figure 12c). Dans l'ensemble, ces résultats démontrent que les effets transgénérationnels liés à l'absence d'une mère peuvent être présents dans les espèces où les soins parentaux ne sont pas obligatoires pour la survie des juvéniles. Nous en concluons que ces effets pourraient être un moteur important dans le maintien de la vie de famille chez ces espèces et plus généralement dans le maintien de la vie de groupe une fois qu'elle a émergé.

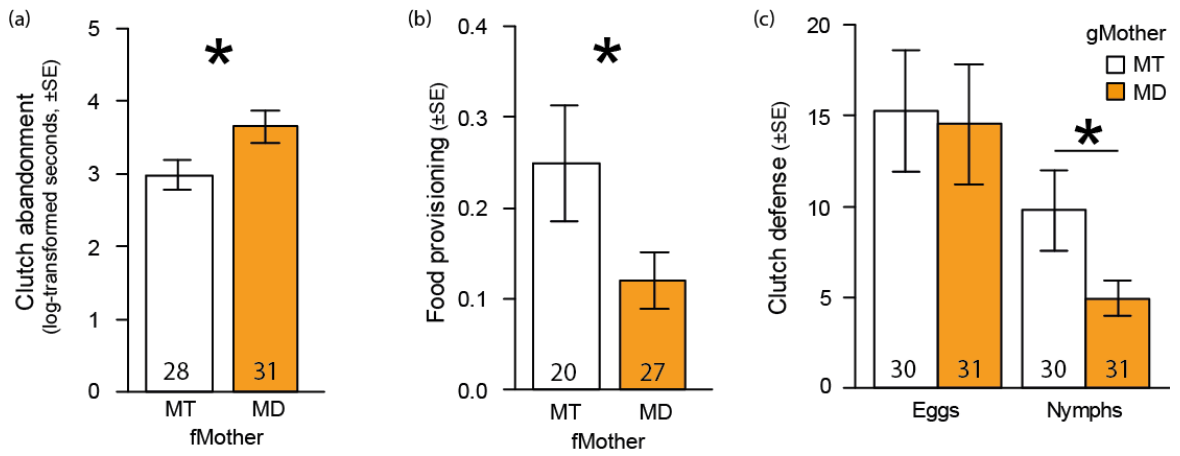

Figure 12 | Effets directs et transgénérationnels de la perte de la mère sur l'expression de trois formes de soins maternels par la génération suivante : (**a**) la durée d'abandon, (**b**) l'approvisionnement en nourriture et (**c**) la défense des juvéniles. MT = élevée avec la mère (maternally tended), MD = élevée sans mère (maternally deprived), fMother = origine de la mère adoptante (foster mother), gMother = origine de la mère génétique des juvéniles adoptés (genetic mother). Les tailles d'échantillons sont au bas de chacune des barres. * P < 0.05. Illustration adaptée de Thesing *et al.*, (2015).

3.1.3 Des bénéfices contre la dépression de consanguinité ?

- **Meunier J** & Kölliker M (2013). *Journal of Evolutionary Biology*, 26(10), 2209–20.

Les soins parentaux sont traditionnellement connus pour neutraliser les effets négatifs d'un grand nombre de stress sur leurs juvéniles, comme par exemple la privation de nourriture, la prédation, le parasitisme ou les variations climatiques (Royle *et al.*, 2012). De façon intéressante, ces stress sont souvent connus pour exacerber les effets négatifs de la consanguinité (appelés dépression de consanguinité) induits par l'accouplement de deux individus génétiquement apparentés (Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999). En limitant les stress subis par les juvéniles, les soins parentaux pourraient donc aider à limiter les effets de la dépression de consanguinité (Avilés & Bukowski, 2006) – un bénéfice qui n'avait jamais été directement testé au moment de notre étude. Nous avons donc testé cette prédiction chez le forficule européen. Pour ce faire, nous avons accouplé des individus génétiquement apparentés (frères et sœurs) ou non-apparentés, et les avons ensuite maintenus avec leur mère (traitement avec soins maternels) ou sans leur mère (traitement sans soin maternel). Nos prédictions étaient que les effets de la dépression de consanguinité sur le développement et/ou la survie des descendants seraient plus forts en l'absence de la mère. Nos résultats ont montré que la dépression de consanguinité - si elle est présente - n'affectait pas ces deux traits chez *F. auricularia* et donc *a fortiori*, que son expression ne dépendait pas de la présence ou l'absence de soins maternels (**Meunier & Kölliker, 2013**). Il est tout de même intéressant de noter que l'absence de dépression de consanguinité révélée par nos résultats suggère qu'il n'y a pas d'évitement de consanguinité dans la population étudiée et donc que celle-ci a été purgée de ses allèles récessifs hautement délétères, c'est-à-dire ceux s'exprimant dans les premières étapes de la

vie (Glémin, 2003). Il s'agit d'un point important, car plusieurs études suggèrent que la dispersion des adultes est très faible chez cette espèce (Moerkens *et al.*, 2010) et donc que les opportunités d'accouplements entre individus apparentés peuvent être fréquentes dans la nature. A la suite de notre étude, l'hypothèse que nous avions soulevé a été testée chez un autre insecte, le scarabée nécrophore *Nicrophorus vespilloides*. Comme nous l'avions alors prédit, Pilakouta *et al.* (2015) ont démontré chez cette espèce que les soins maternels permettent bien de limiter la dépression de consanguinité et ont donc confirmé l'existence d'une source de bénéfices peu connue des soins parentaux.

3.1.4 Les bénéfices pour les juvéniles sont-ils conditions-dépendants ?

- **Meunier J** & Kölliker M (2012). *Biology Letters*, 8(4), 547–550.
- Kramer J, Körner M, Diehl JMC, Scheiner C, Yüksel-Dadak A, Christl T, Kohlmeier P & **Meunier J** (2017). *Functional Ecology*, in press.

L'évolution des soins parentaux implique que leur expression apporte plus de bénéfices que de coûts pour les parents. Alors que beaucoup d'études se sont intéressées à l'importance des conflits génétiques et de la variation d'apparentement entre les membres d'une même famille sur ce ratio coût/bénéfice (Clutton-Brock, 1991; Mock & Parker, 1997; Ratnieks *et al.*, 2006; Royle *et al.*, 2012), le rôle des conditions environnementales et surtout de l'apport en nourriture sur l'évolution des soins parentaux ont reçu peu d'attention (Wong *et al.*, 2013). Une hypothèse traditionnelle est que les conditions environnementales difficiles favorisent l'émergence des soins parentaux du fait des bénéfices significatifs qu'ils apportent alors pour les juvéniles (Wilson, 1975). Dans de telles conditions, les juvéniles élevés par leurs parents devraient donc être mieux aptes à survivre que ceux n'ayant pas accès aux soins parentaux. Mais les conditions environnementales difficiles peuvent aussi exacerber les coûts de ces soins pour les parents et ainsi réduire (pour les parents) la valeur des juvéniles présents au détriment de ceux potentiellement produits dans le futur (Trivers, 1974; Klug & Bonsall, 2007). Il est donc possible de prédire que les juvéniles élevés dans des conditions environnementales difficiles ne devraient pas mieux survivre en présence qu'en l'absence de leurs parents. Nous avons testé ces deux prédictions chez le forficule européen (**Meunier & Kölliker, 2012b**). En

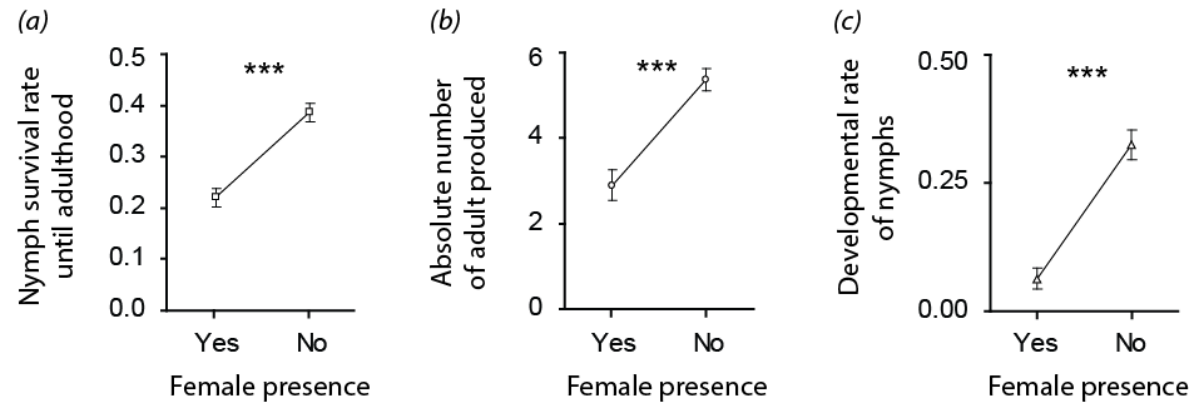

Figure 13 | Effets de la présence de la mère lorsque la nourriture est limitée sur (**a**) la survie, (**b**) le nombre et (**c**) la vitesse de développement des juvéniles jusqu'à l'âge adulte. *** P < 0.001. Figure tirée de Meunier & Kölliker (2012b).

particulier, nous avons élevé des juvéniles avec ou sans leur mère, tout en leur offrant un accès limité à la nourriture. De façon surprenante, nos résultats ne supportent aucune des deux prédictions et révèlent plutôt que la présence de la mère réduit la survie des juvéniles (Figure 13). Ce résultat est à mettre en perspective avec un résultat précédent, montrant que la présence des mère augmente la survie des juvéniles jusqu'au dernier stade larvaire lorsque ces derniers ont un accès *ad libitum* à la nourriture (Kölliker, 2007). Nos résultats montrent donc non seulement que la présence de la mère n'est pas forcément associée à des bénéfices pour les juvéniles, mais aussi qu'elle est potentiellement associée à des coûts pour les juvéniles (voir aussi notre étude détaillée plus haut mais dans des conditions optimales ; Thesing *et al.*, 2015). Il semblerait donc que les mauvaises conditions environnementales ne puissent pas être considérées comme une pression de sélection universelle favorisant l'émergence et le maintien de la vie de famille (Wilson, 1975; Wong *et al.*, 2013).

Pourquoi la présence de la mère diminue-t-elle la survie des juvéniles lorsque la quantité de nourriture disponible est limitée ? L'hypothèse la plus probable est que la mère et les juvéniles entrent en compétition pour l'accès à cette nourriture. Nous avons testé cette hypothèse au travers d'une nouvelle expérience (**Kramer *et al*, 2017**). Son protocole expérimental fut le même que pour l'expérience précédente (Meunier & Kölliker, 2012b) sauf que nous avons mesuré ici 1) la prise de poids de la mère et des juvéniles pendant la vie de famille, 2) le poids initial de chacune des parties (qui est un indicateur de leur qualité générale) et enfin 3) l'investissement des mères dans leur seconde reproduction. A l'aide de ces mesures, nous avons d'abord voulu comprendre si une compétition avait effectivement lieu entre les deux parties en testant si la consommation de nourriture par la mère se faisait au détriment de la survie des juvéniles. En accord avec cette prédiction, nos résultats ont montré que la prise de poids des mères affecte négativement le taux de survie des juvéniles jusqu'à l'âge adulte (Figure 14a). Il est important de noter que cette association n'était présente que lorsque la mère et les juvéniles étaient ensemble – ce qui permet d'éliminer la possibilité que ce pattern ne reflète qu'un lien intrinsèque (par exemple génétique) dans la capacité des mères et celle de leurs juvéniles à prendre du poids au cours des interactions familiales. De façon intéressante, cette association révèle que certaines femelles prennent plus de poids que d'autres, et donc que le niveau de compétition entre mère et juvéniles peut être variable entre familles.

Qu'est-ce qui détermine le niveau de cette compétition ? Pour répondre à cette question, nous avons cherché à associer la prise de poids des mères (et des juvéniles) à leur poids initial, un paramètre connu pour refléter leur qualité (Meunier *et al.*, 2012; Koch & Meunier, 2014). Nos résultats confirment l'existence de ce lien et révèlent que la prise de poids des mères est négativement corrélée à leur poids initial (Figure 14b). Il semblerait donc que plus les femelles sont de bonne qualité, plus elles laissent leurs juvéniles avoir un accès aux ressources. En d'autre termes, la compétition (c'est-à-dire une prise de poids conséquente de la mère) n'intervient que lorsque les mères sont de mauvaise qualité. Est-ce que cette compétition apporte bien des bénéfices à ces mères, par exemple sur leur reproduction future ? Nos résultats confirment partiellement nos prédictions, mais montrent que la nature de l'association entre prise de poids de la mère et investissement dans la ponte

future dépend aussi de la prise de poids des juvéniles. En particulier, la prise de poids des mères est positivement associée à leur production d'une deuxième couvée lorsque les juvéniles prennent du poids, mais négativement lorsque les juvéniles prenaient peu de poids (Figure 14c). Tous nos résultats suggèrent donc que la compétition mère-juvénile n'est pas toujours associée à un changement d'investissement de la mère entre sa couvée présente et future (Klug *et al.*, 2012), et que d'autres paramètres pourraient favoriser son expression. Par exemple, son expression pourrait reposer sur le maintien d'une bonne condition générale de la mère afin d'assurer les soins (Bateson, 1994) : certaines femelles pourraient avoir lourdement investi dans leur couvée actuelle (bien que de faible qualité), donnant lieu à une association négative entre gain de poids des mères et probabilité de produire une seconde ponte dans les couvées avec un faible gain de poids des juvéniles. Ce scenario suggère que le conflit entre mère et juvéniles pour l'accès aux ressources (mais pas la compétition !) pourrait

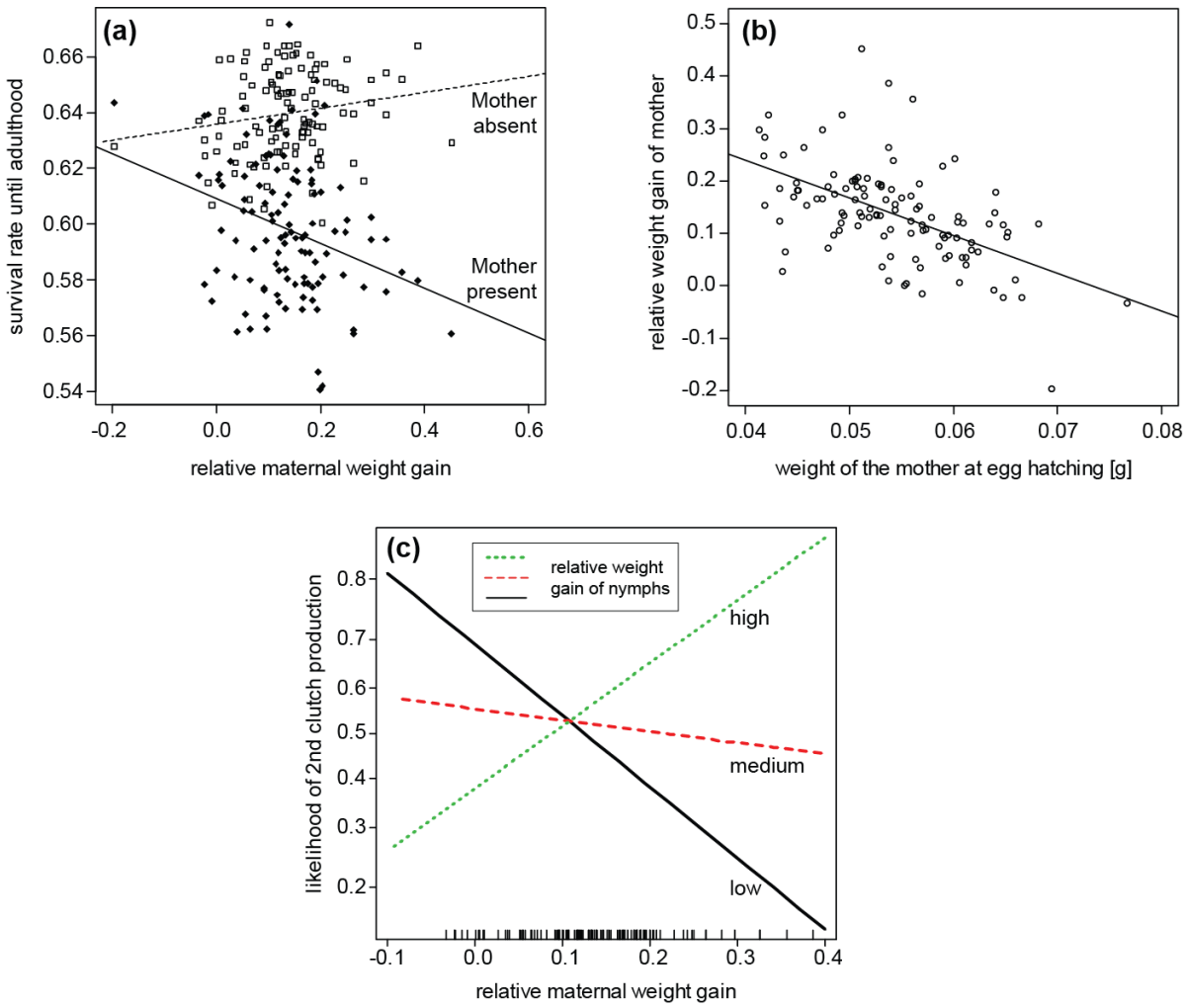

Figure 14 | Compétition mère-juvéniles pour l'accès à la nourriture chez le forficule européen. (**a**) La prise de poids de la mère est négativement corrélée au taux de survie des juvéniles jusqu'à l'âge adulte, mais uniquement lorsque la mère est présente. (**b**) La prise de poids des mères pendant la vie de famille est négativement corrélée à leur poids au début de cette vie de famille. (**c**) La prise de poids de la mère pendant la vie de famille est associée à ses chances de produire une deuxième ponte, mais la nature de cette association dépend de la prise de poids des juvéniles pendant cette vie de famille. Figure adaptée de Kramer *et al*, 2017.

être limité si les mères réinvestissent dans leur couvée actuelle les ressources pourtant acquises de façon compétitive : ce phénomène réduisant le coût de cette compétition pour ces juvéniles.

3.2 La coopération entre juvéniles

- Falk J, Wong JWY, Kölliker M **& Meunier J** (2014). *The American Naturalist, 183(4), 547–557.*
- Kramer J, Thesing J & **Meunier J** (2015). *Journal of Evolutionary Biology, 28(7), 1299–1308.*
- Kramer J & **Meunier J** (2016). *Behavioral Ecology*, 27(2), 494–500.
- Körner M, Diehl JM & **Meunier J** (2016) *Behavioral Ecology*, 27 (6):1775-1781.

Pendant la vie de famille, les interactions comportementales entre juvéniles sont généralement considérées comme étant de nature compétitives et aller jusqu'au combat létal (Mock & Parker, 1997). De ce fait, il est traditionnellement admis que les seuls bénéfices que les juvéniles reçoivent des interactions familiales viennent des soins prodigués par leurs parents et donc que ces soins constituent le promoteur principal de l'évolution de la vie de famille du point de vue des descendants (Clutton-Brock, 1991; Klug *et al.*, 2012; Royle *et al.*, 2012). Pourtant, la coopération entre descendants est un phénomène commun, voire obligatoire, chez de nombreuses espèces animales. Chez certains oiseaux et poissons, par exemple, les descendants devenus adultes restent avec leurs parents et les aident alors à élever la nouvelle génération en prodiguant des soins à ces derniers – une forme de coopération envers des frères/sœurs alors à l'état d'œufs et de juvéniles (*cooperative breeding*; Hatchwell & Komdeur, 2000; Cant, 2012; Zöttl *et al.*, 2013). Dans les colonies d'insectes eusociaux telles que les fourmis ou les termites, les ouvrières/ouvriers adultes sont les seul(e)s responsables du développement du couvain produit par la mère et expriment donc des formes de coopération envers leurs frères/sœurs alors à l'état d'œufs et de larves (Wilson, 1971; Bignell *et al.*, 2011). De façon intéressante, quelques études montrent aussi que la coopération sous forme d'échanges de nourriture peut être exprimée de juvénile à juvénile (Forbes, 2007; Roulin & Dreiss, 2012). Cette forme de coopération se retrouve dans les familles de la chouette effraie *Tyto alba*, chez qui les juvéniles partagent les proies apportées par les parents en usant de méthodes de négociations vocales sophistiquées (Roulin *et al.*, 2000, 2016; Johnstone & Roulin, 2003; Dreiss *et al.*, 2010, 2016). Elle se retrouve aussi chez la mouette rieuse *Chroicocephalus ridibundus*, chez qui les juvéniles coordonnent leur demande de nourriture envers les parents et arrivent ainsi à obtenir un meilleur investissement parental (Mathevon & Charrier, 2004). Enfin, la coopération entre juvéniles a été démontrée chez le Fou à pieds bleus *Sula nebouxii*, un oiseau chez qui les juvéniles dominants peuvent laisser un accès à la nourriture à leurs subordonnés (alors que le cannibalisme entre juvéniles est fréquent chez cette espèce) et limite ainsi les coûts potentiellement associés à l'expression d'une compétition agressive pour ces derniers (Anderson & Ricklefs, 1995).

Les quelques exemples de coopération entre juvéniles reportés plus haut sont importants, car ils révèlent que les interactions entre juvéniles ne sont pas uniquement coûteuses et risquées pour ces derniers. Mais ces exemples viennent tous d'espèces altriciales chez qui les juvéniles sont peu mobiles et ne peuvent pas survivre en l'absence des

parents. On peut donc se demander si la coopération entre juvéniles est 1) un phénomène qui a émergé après que la vie de groupe soit devenue obligatoire afin de limiter les coûts de la vie de groupe pour des juvéniles obligés de vivre avec leurs frères et sœurs (comme dans les systèmes altriciaux ou eusociaux) ou au contraire, si elle a est 2) un processus ancestral qui a pu favoriser les premières étapes de l'évolution de la vie de famille en apportant des bénéfices complémentaires (c'est-à-dire des bénéfices additionnels aux soins parentaux) aux juvéniles faisant le choix de rester en famille. Démêler ces deux scénarii évolutifs est primordial si l'on veut mieux comprendre les mécanismes favorisant l'émergence et le maintien de la vie de famille, et plus généralement l'évolution de la vie sociale.

(a)

(b)

Figure 15 | Un juvénile de 1er stade larvaire (a) avec une coloration naturelle et (b) après avoir consommé de la nourriture verte. La coloration du tube digestif se fait en donnant aux juvéniles des boules de pollen mélangées à un colorant alimentaire bleu – ce qui lui donne une couleur verte. Les individus colorés (donneurs) peuvent être mis en contact avec d'autres individus non-colorés (receveurs) pendant 24h. Au bout de ce laps de temps, il est alors possible de voir (à l'œil nu ou avec l'aide d'une loupe binoculaire) si les receveurs sont devenus verts et donc s'il y a eu un échange de nourriture (Staerkle & Kölliker, 2008).

Une manière de démêler ces deux scénarii évolutifs est de chercher si les juvéniles peuvent coopérer dans les espèces où ils ont la capacité de quitter le nid précocement et de survivre en l'absence des parents, comme c'est le cas chez le forficule européen. Entre 2014 et 2016, nous avons donc conduit une série d'expériences afin 1) de déterminer si la coopération entre juvéniles était présente chez le forficule européen et 2) de mieux comprendre les facteurs potentiellement responsables de son niveau d'expression. Nos études se sont principalement focalisées sur l'échange de nourriture, puisque c'est la forme de coopération entre juvéniles la plus connue dans la littérature (mais chez les oiseaux, voir plus haut). Notre première expérience avait pour but de révéler si oui ou non les juvéniles échangeaient de la nourriture entre eux, et si les contacts avec la mère ou simplement sa proximité pouvaient affecter cet échange (**Falk *et al.*, 2014**). Pour ce faire, des groupes de quatre juvéniles ont été rassemblés de sorte à avoir 3 donneurs (avec le contenu de leur tractus digestif expérimentalement coloré en vert – voir Figure 15) et 1 receveur (avec le tractus digestif vide) par groupe. Pour chacun de ces groupes, la mère des juvéniles (avec le tractus digestif vide) était soit mise directement avec eux, soit mise derrière une grille permettant des échanges chimiques mais non tactiles avec les juvéniles, soit mise dans une autre boite et sortie de l'expérience. Au bout de 24 heures, nous avons déterminé pour chaque groupe si le receveur était devenu vert, c'est-à-dire s'il y avait eu un transfert de nourriture de la part des juvéniles donneurs. Nos résultats montrent que des transferts de nourriture ont bien eu lieu dans tous les groupes, mais que ces transferts représentaient 30% des individus testés lorsqu'il y avait des contacts avec la mère, 85% lorsqu'il n'y avait aucun

contact tactile avec la mère, et enfin 70% lorsque la mère était totalement absente (Figure 16A)(**Falk *et al.*, 2014**). En conclusion, nous pouvons dire que la nourriture acquise par les juvéniles est transmise à d'autres juvéniles chez cette espèce, que cet échange de nourriture intervient en présence et en l'absence de la mère, mais aussi que des contacts directs avec la mère tendent à limiter la fréquence de ces échanges.

Notre deuxième expérience avait pour but de déterminer si cet échange de nourriture pouvait être un processus passif qui s'opère par la consommation des fèces produits par les autres juvéniles et/ou s'il s'agissait d'un processus actif, comme la trophallaxie (Suárez & Thorne, 2000; Staerkle & Kölliker, 2008), qui implique des échanges directs entre les juvéniles. Dans ce but, des juvéniles receveurs ont été isolés dans des boites qui contenaient soit des fèces colorés produits par d'autres juvéniles les jours précédents, soit des juvéniles donneurs (avec le contenu de leur tractus digestif coloré en vert), soit rien. Vingt-quatre heures plus tard, nos résultats montrent que près de 75% des juvéniles ayant uniquement accès aux fèces sont devenus colorés, que 90% de ceux ayant été en contact avec des donneurs sont devenus colorés, mais qu'aucun de ceux ayant accès à aucun des deux précédents traitements ne se sont colorés (Figure 16, Falk *et al.*, 2014). Ainsi nous pouvons conclure que le transfert de nourriture entre juvéniles peut se faire (au moins en partie) sans contact direct entre les deux individus au travers de la consommation de fèces, mais que le niveau de ce transfert augmente lorsque les individus ont la possibilité d'avoir un contact avec d'autres juvéniles.

Nous avons enfin réalisé une troisième expérience afin de comprendre comment et pourquoi les contacts entre juvéniles pourraient favoriser le transfert de nourriture. Chez les

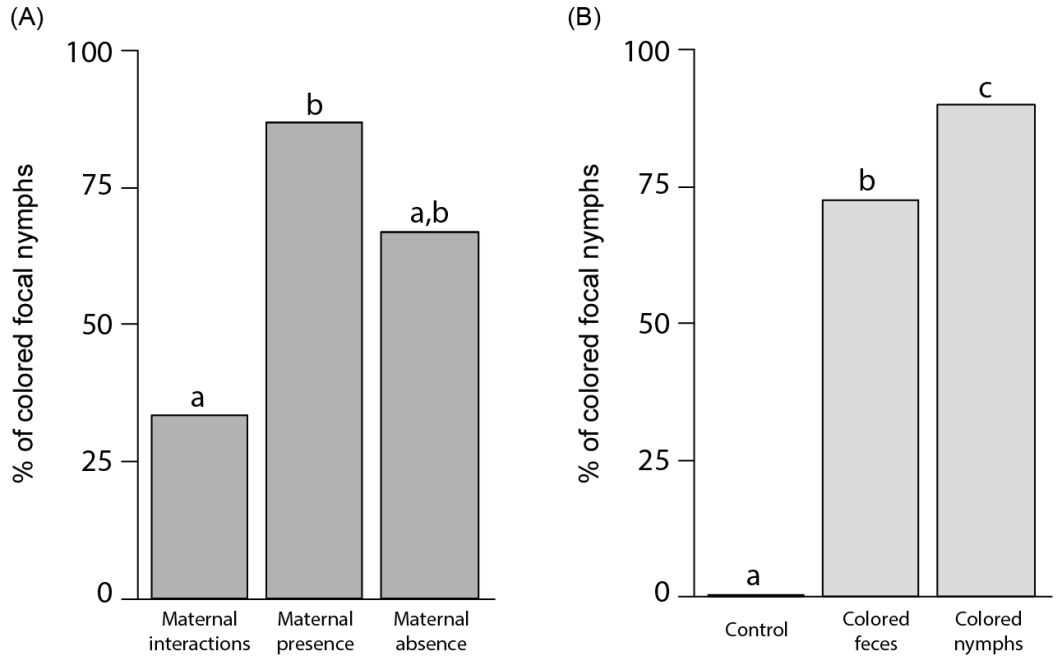

Figure 16 | Pourcentage de groupe expérimentaux dans lesquels le juvénile receveur est devenu coloré après avoir été (**A**) en contact avec leur mère (Maternal interactions), proche de leur mère mais sans contact avec elle (maternal presence) ou sans leur mère (maternal absence) ; et (**B**) seul (control), en contact avec des fèces de juvénile colorés (colored feces) ou en contact avec des juvéniles colorés (colored nymphs) (B). Les lettres différentes illustrent $P < 0.05$. D'après Falk *et al.* (2014).

insectes, trois comportements sont principalement connus pour permettre l'échange de nourriture précédemment ingérée entre deux individus. D'abord, la trophallaxie stomodéale qui est un transfert par contacts bouche-bouche. Ensuite, la trophallaxie proctodéale qui est un transfert par contacts bouche-anus. Enfin, l'allo-coprophagie, qui consiste à consommer les fécès produits par les membres du groupe. Pour tester l'expression et l'importance relative de ces différents comportements sur l'échange de nourriture entre juvéniles chez le forficule, nous avons filmé des paires d'individus constituées d'un juvénile donneur et d'un receveur. Dans le but de tester aussi si cet échange dépend de l'apparentement génétique entre les deux individus (apparentés ou non-apparentés) et du besoin nutritionnel du receveur (affamé ou bien nourri), ces deux facteurs ont aussi été manipulés de façon croisée (**Falk *et al.*, 2014**). Nos résultats révèlent que le transfert de nourriture se fait selon deux processus qui dépendent de l'apparentement mais pas du besoin nutritionnel des juvéniles. Lorsqu'ils sont apparentés, le transfert se fait par allo-coprophagie, c'est-à-dire que les receveurs consomment plus de fécès des donneurs (Figure 17a). Lorsqu'ils sont non-apparentés, le transfert de nourriture se fait par trophallaxie stomodéale c'est-à-dire que les receveurs viennent chercher la nourriture directement à l'arrière des donneurs (Figure 17b). La surconsommation de fèces parmi les juvéniles apparentés reflète ce qui serait attendu si ces fèces étaient un bien public (comme c'est le cas des sidérophores chez certaines bactéries, voir Griffin *et al.*, 2004; Diggle *et al.*, 2007) et dont la production bénéficierait à tous les membres (apparentés) de la famille. Cette hypothèse de bien public est aussi supportée par le fait que l'absence de surproduction de fèces dans les paires non-apparentées est associée à l'expression d'une trophallaxie proctodéale, un comportement qui pourrait traduire un conflit pour l'acquisition et la monopolisation de ce bien commun dans les paires non-apparentées. De façon intéressante, nos résultats montrent aussi que les besoins

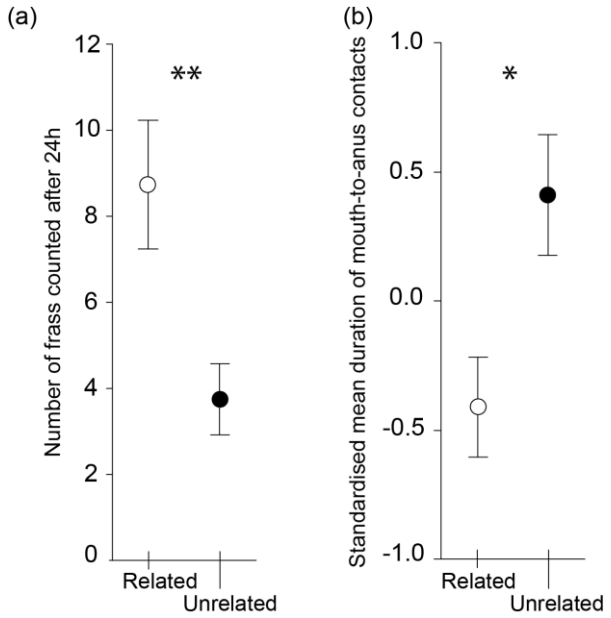

Figure 17 | Effets de l'apparentement génétique entre juvéniles donneurs et receveurs sur (**a**) le nombre de fécès produits par les donneurs après 24h et (**b**) sur la durée moyenne des contacts bouche-anus. D'après Falk *et al.* (2014).

nutritionnels du receveur n'influencent en rien l'échange de nourriture (**Falk *et al.*, 2014**). Ce résultat suggère que le rôle nutritif de l'allo-coprophagie pourrait être limité chez les forficules. A l'inverse, ce comportement pourrait apporter d'autres types de bénéfices, tels que 1) la digestion de particules difficiles à dégrader en un seul passage dans le tube digestif et qui pourraient être des sources importantes de protéines, lipides ou carbohydrates (Martin & Reddy, 1984; Nalepa *et al.*, 2001) ou 2) le transfert de composés immunitaires et/ou de la flore intestinale au sein des membres de la famille (Pellens *et al.*, 2007; Bignell, 2011; Kaltenpoth & Engl, 2014).

Afin de mieux comprendre les potentiels bénéfices nutritifs associés à la consommation de fèces, nous avons conduit une nouvelle expérience dans laquelle nous avons suivi la survie de juvéniles ayant uniquement accès à des fèces de juvéniles apparentés, des fèces de leur mère ou rien du tout. Pour déterminer si l'apport nutritif de ces fèces est spécifique (c'est-à-dire ne peut pas être obtenu par de la nourriture standard), nous avons aussi ajouté deux traitements dans lesquels les juvéniles avaient accès à de la nourriture standard seule ou à de la nourriture standard avec des fèces de juvéniles (**Körner *et al.*, 2016**). Nos résultats montrent que l'accès à des fèces de juvénile rallonge significativement l'espérance de vie des consommateurs, alors que l'accès à des fèces de mère entraine une mort aussi rapide qu'en l'absence de tout type de ressource (Figure 18). Pour autant les fèces de juvéniles ne permettent pas aux individus de survivre plus de 23 jours, contrairement à la nourriture standard qui permet à une majorité de juvéniles de survivre au-delà de 25 jours, et ce avec ou sans fèces de juvénile (Figure 18). Ces résultats démontrent donc que l'allo-coprophagie peut apporter un bénéfice important pour les juvéniles en l'absence d'autre source de nourriture. Le fait que ces bénéfices ne soient pas présents avec les fèces de la mère pourrait simplement refléter la meilleure digestion des adultes par rapport aux juvéniles (Engel & Moran, 2013), et donc la plus haute valeur nutritive des fèces produits par ces derniers.

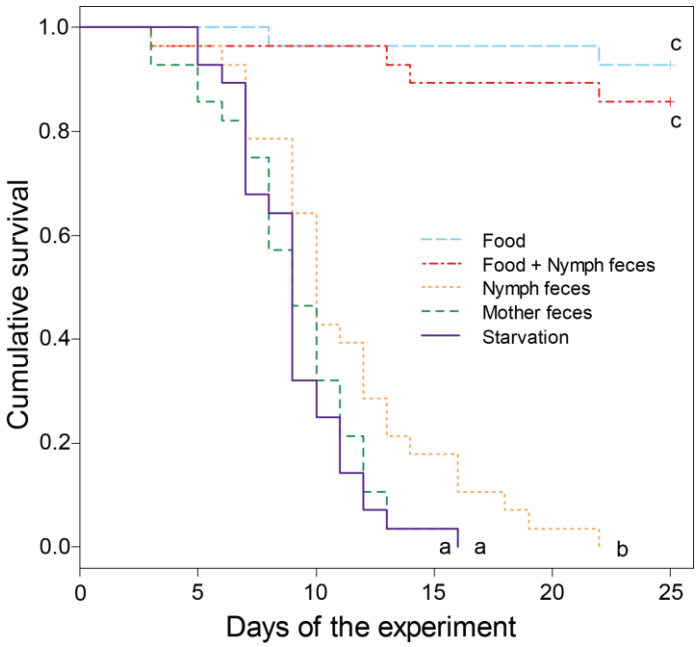

Figure 18 | Effet de l'accès à la nourriture standard et/ou aux fèces sur la survie des juvéniles. Chaque ligne représente la survie cumulée des juvéniles ayant eu accès soit à de la nourriture standard (*Food*; Taux de survie médian, LT50 ± SE = 49.8 ± 7.7), soit aux fèces produits par des juvéniles (*Nymph feces*, LT50 = 15.6 ± 0.3), soit à des fèces produits par leur mère (*Mother feces*, LT50 = 40.7 ± 3.4), soit à de la nourriture standard et des fèces produits par des juvéniles (*Food + Nymph feces*, LT50 = 15.6 ± 0.3), soit rien du tout (*Starvation*, LT50 = 13.5 ± 0.2). Les lettres différentes correspondent à P-value < 0.03. D'après Körner *et al.* (2016).

Ayant établi que les juvéniles échangent de la nourriture pendant la vie de famille, nous nous sommes posés la question des liens qu'il pourrait y avoir entre cette forme potentielle de coopération entre juvéniles et l'expression des soins parentaux tels que l'apport en nourriture. En effet, ces deux processus peuvent *en principe* s'exprimer au même moment pendant la vie de famille et chacun apporter d'importants bénéfices nutritionnels et non-nutritionnels pour les juvéniles. Ces deux processus pourraient donc être indépendants ou associés de façon complémentaire ou compensatoire. Dans le cas de l'association complémentaire, le niveau d'échange de nourriture entre juvéniles devrait augmenter en même temps que le niveau de soins maternels. Ce phénomène est attendu si les coûts liés à la coopération pour les juvéniles diminuent lorsque le niveau de soin parental est haut. Dans le cas d'une association compensatoire, par contre, le niveau d'échange de nourriture entre juvéniles devrait diminuer lorsque le niveau de soin maternel augmente. Ce type d'association est plutôt attendu lorsque les bénéfices liés à la coopération pour les juvéniles augmentent alors que le niveau de soin parental est bas. Pour tester ces hypothèses, nous avons mesuré au sein des mêmes familles, les niveaux de transferts de nourriture entre les juvéniles et entre la mère et les juvéniles, tout en estimant l'importance du nombre de juvéniles présents sur ces échanges (**Kramer *et al.*, 2015**). Nos résultats révèlent d'abord que le taux de transfert de nourriture entre juvéniles augmente avec le nombre de juvéniles présents dans le groupe, alors que ce nombre n'influence pas le taux de transfert de nourriture venant de la mère (Figure 19A). Lorsqu'on contrôle ensuite par le nombre de juvéniles dans le groupe, nous voyons une corrélation significative et négative entre les deux taux de transferts : les juvéniles échangent moins de nourriture lorsque la mère en apporte plus (Figure 19B). Mais est-ce qu'un fort taux d'échange de nourriture entre juvéniles et donc un faible apport de la part des femelles apporte des bénéfices à chacune de ces parties ? Afin de répondre à cette question, nous avons ensuite mesuré la vitesse de développement des juvéniles et leur survie

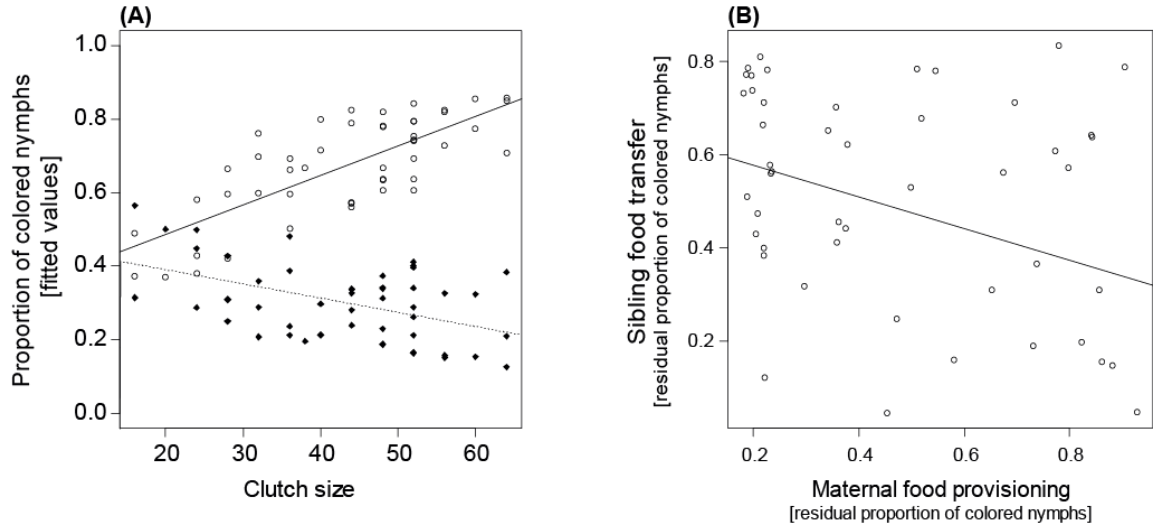

Figure 19 | Association entre coopération entre juvéniles et soins maternels. (**A**) Influence du nombre de juvéniles sur la proportion de nymphes ayant reçu de la nourriture de leur mère (carrés noirs ; t46 = 0.86, P = 0.386) ou de leur frères et sœurs (rond blanc, t46 = 2.43, P = 0.019) ; (**B**) Corrélation entre le niveau d'échange de nourriture entre juvéniles (corrigé par leur nombre) et le niveau de nourriture donnée par la mère (q = -0.306, S46 = 24062, P = 0.035). D'après Kramer *et al.* (2015).

jusqu'à l'âge adulte, ainsi que le nombre d'œufs produits par les femelles dans leur ponte suivante. Nos résultats démontrent que le niveau de transfert de nourriture entre juvénile n'est pas associé à des bénéfices pour les juvéniles en qualité de vitesse de développement et de survie (Kramer *et al.*, 2015). Pour les mères, nous trouvons que ce taux de transfert n'est pas associé à des bénéfices mais, de manière plus surprenante, qu'il est associé à des coûts. En particulier, les mères de juvéniles qui échangent plus de nourriture produisent moins d'œufs dans leur ponte suivante (Kramer *et al.*, 2015) – reflétant probablement la faible qualité de ces femelles (voir plus bas). Dans l'ensemble, ces résultats démontrent une association compensatoire entre ces deux processus qui ne donne pas de plus-value en ce qui concerne la fitness des juvéniles et de la mère. La coopération observée entre juvéniles pourrait donc être est un processus permettant de contrebalancer les effets négatifs d'un faible niveau de soins maternels, voire de ceux liés à l'absence totale d'une mère (comme démontré lorsqu'elles furent expérimentalement retirées des familles dans l'étude précédente; Falk *et al.*, 2014).

Pourquoi est-ce qu'un taux croissant d'échange de nourriture entre juvéniles est associé à un coût pour les mères ? Une réponse pourrait être parce que ce taux reflète négativement la qualité de la mère. En effet, ce taux d'échange est inversement corrélé à l'apport de nourriture par la mère, de sorte que les femelles qui apportent le moins de soins (sous forme de nourriture) sont aussi celles qui investissent le moins dans leur future reproduction. Pour tester cette hypothèse, nous avons élaboré une nouvelle expérience dans laquelle nous avons d'abord manipulé la condition des mères en leur donnant (ou non) un accès à de la nourriture et ensuite mesuré le niveau d'échange de nourriture entre les juvéniles **(Kramer & Meunier, 2016b**). Nos résultats confirment d'abord que ce niveau d'échange augmente avec le nombre de juvéniles (comme révélé précédemment, Kramer *et al.*, 2015) mais révèlent ensuite que cette association est uniquement présente lorsque la mère est en mauvaise condition. La condition de la mère est donc un élément primordial pour expliquer le niveau d'échange de nourriture exprimé entre les juvéniles.

Dans l'ensemble, nos résultats démontrent que l'échange de nourriture entre juvéniles est présent chez le forficule européen. Cet échange se fait par plusieurs moyens directs (trophallaxie proctodéale) et indirects (allo-coprophagie) pour lesquels le choix dépend de l'apparentement génétique (et/ou la familiarité) entre le donneur et le receveur. Cet allo-coprophagie permet d'augmenter la survie des juvéniles en l'absence de nourriture, mais pourrait être associé à d'autres types de bénéfices pour les receveurs tels que des bénéfices immunitaires et/ou des échanges de microbiotes intestinaux. Reste la question des coûts associés à cet échange de nourriture – un paramètre crucial pour le définir comme une forme de coopération. A ce jour, déterminer la présence de ces coûts et les quantifier sont une priorité de mes recherches, mais mesurer ces deux aspects est associé à des difficultés techniques encore difficilement solvables. Indépendamment de la présence ou l'absence de ces coûts, nos résultats démontrent dans l'ensemble que cette forme potentielle de coopération n'est pas un mécanisme qui nécessite un système social permanent et/ou fixe au cours du temps, comme ceux que l'on trouve dans les espèces à reproduction communautaire (*cooperative breeding*) ou eusociales. A l'inverse, ils suggèrent que ce phénomène est un

comportement qui pourrait être ancestral au cours de l'évolution de la vie sociale et qui pourrait donc avoir favorisé l'émergence et la maintenance de la vie de groupe à partir de la vie solitaire. Cette forme de coopération pourrait en effet augmenter l'intérêt des juvéniles à rester avec leur frères/sœurs, ce qui faciliterait l'évolution des soins parentaux et plus généralement les formes plus dérivées de vie sociale.

3.3 La protection collective contre les pathogènes

3.3.1 Immunité sociale et évolution de la vie de groupe

- **Meunier J** (2015). *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 370, 20140102.

Un des coûts majeurs de la vie de groupe repose sur le risque élevé que ses membres soient exposés et/ou infectés par les pathogènes. Ce risque est particulièrement important chez les espèces sociales, car les contacts fréquents entre les individus sont connus pour faciliter la transmission des pathogènes et parce que l'apparentement génétique souvent fort au sein d'un groupe rend ses membres sensibles aux mêmes souches de pathogènes (Pie *et al.*, 2004, 2005; Masri & Cremer, 2014; Stroeymeyt *et al.*, 2014). Pour limiter ce risque, les animaux sociaux ne sont pas uniquement capables d'utiliser leur propre système immunitaire, mais aussi de mettre en place des défenses collectives. Ce phénomène, appelé immunité sociale, comprend de nombreux mécanismes tels que l'utilisation de substances antimicrobiennes comme matériel de nid, le nettoyage réciproque des individus (allogrooming) ou l'évacuation des corps vers l'extérieur du nid (Figure 20 ; Cremer et al., 2007). Depuis une dizaine d'années, les études sur l'immunité sociale se sont principalement intéressées aux insectes eusociaux, tels que les fourmis, les termites et certaines abeilles (Schmid-Hempel, 1998; Cremer *et al.*, 2007; Wilson-Rich *et al.*, 2009b; Hamilton *et al.*, 2011; Rosengaus *et al.*, 2013), négligeant *de facto* nos connaissances sur son expression et son importance dans les espèces à la vie de groupe temporaire et facultative (Cotter & Kilner, 2010). S'intéresser à ces espèces est pourtant primordial si l'on veut mieux comprendre le lien entre vie de groupe et pathogènes, et plus généralement le rôle des pathogènes dans l'émergence et le maintien de la vie de groupe à partir d'un état solitaire. Je me suis donc intéressé à cette question dans une review publiée en 2015 (**Meunier, 2015**). Dans cette dernière, je mets d'abord en avant le fait qu'il faut introduire une définition claire de ce qu'est et ce qui n'est pas de l'immunité sociale. Une telle définition est cruciale, car celles disponibles au moment de l'écriture de cette étude ne permettaient pas clairement de différencier l'immunité sociale de l'immunité personnelle. De ce fait, la littérature regorge d'exemples dans lesquels un mécanisme servant principalement à la protection personnelle d'un individu est défini comme une forme d'immunité sociale, car en protégeant cet individu il protège aussi indirectement les autres membres de la colonie. La limite d'une telle définition est qu'elle inclut tous les processus immunitaires existant comme des formes d'immunité sociale. En m'inspirant d'une réflexion développée par Cotter & Kilner (2010), j'ai donc proposé de redéfinir l'immunité sociale comme « tout mécanisme individuel ou collectif qui émerge et/ou est maintenu au moins en partie grâce à son rôle de

protection contre les parasites envers les autres membres du groupe ». Mais alors que cette définition apporte plus de clarté sur ce que l'immunité sociale est ou n'est pas, elle rend aussi difficile toute démonstration empirique qu'un processus est bien une forme d'immunité sociale. Cette difficulté repose sur le fait qu'il faudrait pouvoir démontrer que les bénéfices collectifs d'un mécanisme de protection prévalent sur ses bénéfices individuels, ce qui est particulièrement difficile à tester dans les espèces où la vie de groupe est permanente et obligatoire. Or, la grande majorité des études sur l'immunité sociale se focalisent sur de telles espèces (Schmid-Hempel, 1998; Cremer *et al.*, 2007; Wilson-Rich *et al.*, 2009b; Hamilton *et al.*, 2011; Rosengaus *et al.*, 2013). Cette définition met donc une nouvelle fois en avant l'importance d'utiliser les espèces où la vie de groupe est temporaire et/ou facultative comme modèles biologiques dans l'étude de l'immunité sociale.

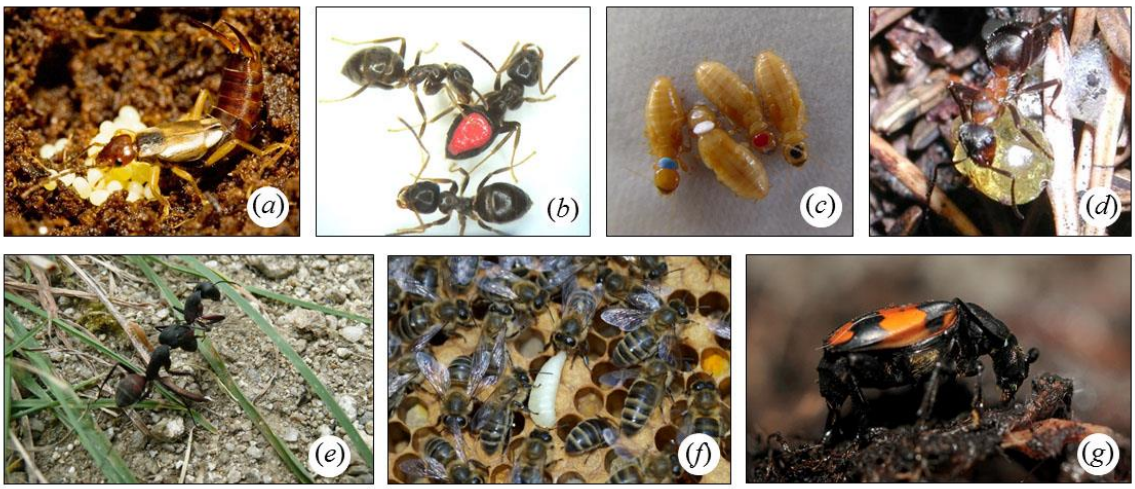

Figure 20 | Exemples d'immunité sociale chez les insectes sociaux et subsociaux. (**a**) Une femelles du forficule européen *F. auricularia* nettoyant ses oeufs couverts de spores (photo: J. Meunier), (**b**) Une ouvrière de la fourmi *Lasius neglectus* nettoyant une autre ouvrière colorée en rouge et préalablement exposée à un champignon entomopathogène (photo: M. Konrad), (**c**) Chez le termite *Zootermopsis angusticollis*, les ouvriers expriment un comportement d'alarme pour prévenir de la présence de pathogène dans le nid (photo: R. Rosengaus), (**d**) Une ouvrière de la fourmi des bois *Formica paralugubris* utilise de la résine comme materiel du nid comme protection contre les pathogènes (photo: A. Maeder), (**e**) Une ouvrière de la fourmi *Camponotus* sp. transporte une ouvrière morte loin du nid (photo: L. Diez), (**f**) Des ouvrières de l'abeille *Apis mellifera* enlèvent une larve malade de la ruche (photo: G. Bigio), (**g**) Une femelle du scarabée nécrophore *N. Vespilloides* utilisant les propriétés antimicrobiennes de ses fèces pour protéger son nid contre les pathogènes (photo: O. Krueger). Illustration tirée de Meunier (2015).

Ayant mis en avant les limites de la définition de l'immunité sociale dans la littérature, je me suis ensuite demandé s'il était possible de trouver des formes « classiques » d'immunité sociale chez les espèces non-eusociales. Comme attendu, les résultats de ma recherche bibliographique montrent que de nombreuses formes d'immunité sociale (selon l'ancienne définition) se retrouvent non seulement chez les espèces eusociales, mais aussi chez des espèces sociales (mais non-eusociales) et même chez des espèces solitaires ! C'est le cas par exemple de l'évitement des habitats contaminés, de l'évitement de la nourriture infectée ou encore de l'expulsion des déchets de l'habitat (Meunier, 2015). D'autres formes d'immunité

sociale « classiques » et impliquant des interactions entre plusieurs individus ne sont – par définition – pas présentes chez les espèces solitaires, mais se retrouvent quelquefois chez les rares études sur des espèces sociales mais non-eusociales. Il s'agit ici, par exemple, de l'allogrooming ou encore de l'application de sécrétions antimicrobiennes sur le couvain (Meunier, 2015). Le dernier point soulevé dans cette review montre notre manque flagrant de connaissances sur le lien entre immunité personnelle et immunité sociale. A ce jour, très peu d'études se sont en effet intéressées à ce lien qui est pourtant crucial dans la défense contre les pathogènes et donc dans l'évolution de la vie de groupe (voir la partie 4.3 de ce mémoire pour une discussion plus élaborée sur ce sujet). Dans l'ensemble, cette review démontre l'importance de commencer à étudier l'immunité sociale chez les espèces non-eusociales afin de pouvoir mieux comprendre si l'immunité sociale a permis l'émergence et le maintien de la vie sociale à partir des formes solitaires, ou si c'est un processus qui a principalement dérivé des formes sociales complexes.

3.3.2 Protéger ses œufs contre les pathogènes

- Boos S, **Meunier J**, Pichon S & Kölliker M (2014). *Behavioral Ecology, 25(4), 754–761.*

Alors que la présence des parents auprès des œufs est souvent connue pour offrir une protection de ces derniers contre la prédation inter- ou intra-spécifique (Miller *et al.*, 2011; Meunier *et al.*, 2012; Trumbo, 2012), on ne sait que très peu de choses du rôle de la présence des parents contre l'infection de leurs œufs par des pathogènes. Ce rôle a été suggéré dans plusieurs espèces d'insectes (Costa, 2006; Trumbo, 2012), mais seules quelques études ont exploré cette question par des approches expérimentales. C'est le cas de deux études sur les dermaptères *Euborellia annulipes* et *Anisolabis maritima,* dans lesquelles il a été montré que l'absence de la mère auprès des œufs augmente significativement les chances que ces œufs meurent et moisissent (Klostermeyer, 1942; Miller & Zink, 2012). Mais les conclusions de ces études ont quelques limites. Par exemple, elles ne permettent pas de savoir si l'effet reporté est simplement dû à la mort des œufs (qui sont ensuite recouverts par les moisissures) ou s'il est dû au fait que les mères enlèvent activement les spores des œufs. De même, il est difficile de savoir si les mères ne font qu'enlever les spores ou si elles apportent aussi d'autres types de protection qui pourraient aider à lutter contre les spores de moisissure. Afin de répondre à ces deux questions, nous avons conduit deux expériences sur le forficule européen (**Boos *et al.*, 2014**). Dans la première expérience, nous avons étudié si la présence d'une mère permet de protéger les œufs contre la moisissure. Pour ce faire, nous avons manipulé dans un design 2x2 croisé la présence de la mère auprès des œufs ou non, et la présence de spores sur les œufs ou non. Comme l'on pouvait s'y attendre, nos résultats démontrent que la présence d'une mère augmente le succès d'éclosion des œufs, que cet effet devient plus fort lorsque les œufs sont préalablement couverts de spores et que la présence de spores sur les œufs entraine un comportement de nettoyage des œufs plus long de la part de la mère (Figure 21a et 21 b). Dans la deuxième expérience, nous avons ensuite testé l'hypothèse selon laquelle les femelles ne faisaient pas qu'enlever les spores, mais déposaient aussi des substances aux propriétés antimicrobiennes (ici des hydrocarbures; voir Blomquist & Bagnères, 2010). Pour ce faire, nous avons mesuré l'évolution de la quantité de ces hydrocarbures (CHC) sur des œufs qui

étaient mis en contact ou isolés de leur mère. Nos résultats montrent que la masse totale de CHC présents sur les œufs reste constante en présence de la mère, mais qu'elle diminue lorsque la mère est absente (Figure 21c). La mère applique donc en effet ces composés sur la surface de ses œufs.

Dans l'ensemble, les résultats de cette étude démontrent que le comportement maternel de nettoyage des œufs sert à la fois à enlever les spores sur ses œufs et à y déposer des composés chimiques par la salive. Le premier mécanisme pourrait constituer une réponse rapide de la mère contre un danger immédiat (c'est-à-dire les spores déjà présentes sur les œufs), alors que le second pourrait plutôt constituer une protection sur le long terme afin d'empêcher les spores d'adhérer et/ou de germer sur les œufs. Il est toutefois important de noter que les CHC ne sont pas uniquement impliqués dans la défense contre les microbes, mais qu'ils sont aussi très importants dans la protection contre la dessiccation et la reconnaissance entre individus chez les espèces sociales (Blomquist & Bagnères, 2010).

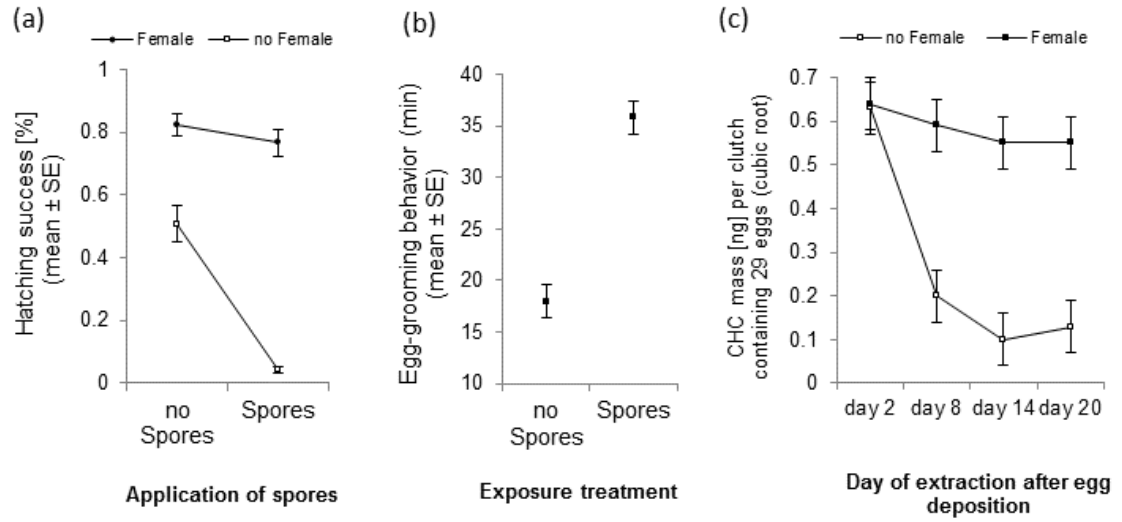

Figure 21 | Effets de la présence de la mère sur la protection des œufs contre les microorganismes. Ces effets sont estimés au travers (a) de l'effet de cette présence sur le succès d'éclosion des œufs qui ont été exposés ou non à des spores de moisissure. (b) de l'effet de la présence des spores sur le temps passé à nettoyer les œufs et (c) de l'évolution de la quantité totale d'hydrocarbures sur les œufs en fonction de la présence de la mère. Figures adapté de Boos *et al.* (2014).

3.3.3 Protéger ses juvéniles contre les pathogènes

- Vogelweith F, Körner M, Foitzik S & **Meunier J** (2017). *BMC Evolutionary Biology*, 17:69.
- Kohlmeier P, Dreyer H & **Meunier J** (2015). *J Insect Physiology.*

Ayant démontré que les mères du forficule européen protègent leurs œufs contre les spores de champignons, nous nous sommes demandés si cette protection avait aussi lieu après l'éclosion des œufs. Cette question est importante, car des vertébrés aux invertébrés, plusieurs facteurs sont souvent avancés pour expliquer l'investissement des juvéniles dans leur propre niveau d'immunité : le risque général d'infection, leur âge et leur accès à une protection extérieure telle que celle proposée par l'immunité sociale (Siva-jothy *et al.*, 2005). Par exemple, il a été démontré que le niveau basal d'immunité des géospizes

fuligineux *Geospiza fuliginosa* (un oiseau) augmente en même temps que la prévalence de parasite dans leur population (Lindström *et al.*, 2004), que les juvéniles de l'abeille à miel améliorent leur investissement dans l'immunité lors de leur développement (Wilson-Rich *et al.*, 2009a) ou encore, que les soins parentaux améliorent la réponse immunitaire des juvéniles chez l'hirondelle rustique *Hirundo rustica* (Saino *et al.*, 1997). Pour autant, savoir si ces facteurs agissent seuls ou en interaction sur l'immunité des juvéniles restait peu compris au moment de notre étude. Pour élucider cette question, nous avons conduit une expérience visant à tester simultanément l'effet de ces trois facteurs sur l'investissement des juvéniles de forficule dans plusieurs traits de la réponse immunitaire (**Vogelweith *et al.*, 2017**). Pour ce faire, des familles expérimentales ont été mises en place dans lesquelles nous avons manipulé de façon croisée la présence de la mère (présente/absente) et la présence de spores de *M. brunneum* dans l'environnement (présence/absence) pendant les 14 premiers jours de la vie

Tableau 2 | Une multitude de méthodes existe dans la littérature pour estimer le niveau d'activité de la phénoloxidase chez les insectes**.** Afin de comprendre les avantages et inconvénients de chacune de ces méthodes et ainsi déterminer la plus robuste, nous avons comparé 3 jeux de données en utilisant chacune de ces méthodes (D1 = Le forficule européen *F. auricularia*, D2 = la fourmi *Cataglyphis cursor* et D3 = le scarabée *Tribolium molitor*). Nos résultats ont montré que les comparaisons pouvaient aller dans des sens différents suivant les méthodes utilisées (voir ci-dessous), appelant au développement d'une nouvelle méthode robuste d'analyse. C'est ce que nous avons fait en mettant en place la méthode PO-CALC, qui est gratuite et disponible sous la forme d'un script R et d'un logiciel autnome. Tableau tiré de Kohlmeier *et al.* (2015)

Type	Potential limitations	Description	Mean PO activity			Example of reference
			D1	D2	D3	
Time difference	• Sensitive to outliers • Depends on the saturation of the chemical reaction	$Abs_{t15} – Abs_{t0}$	0.094	0.100	0.421	(Cotter *et al.*, 2004)
		$Abs_{t10} – Abs_{t0}$	0.042	0.063	2.261	(Demuth *et al.*, 2012)
		$Abs_{t20} – Abs_{t0}$	0.148	0.131	6.084	(Adamo, 2004)
		$Abs_{t15} – Abs_{t5}$	0.085	0.080	3.887	(Srygley *et al.*, 2009)
		$Abs_{t16'200} – Abs_{t0}$	0.487	0.204	12.368	(Mucklow & Ebert, 2003)
Single point	• Sensitive to curves with a delayed increase • Sensitive to noisy graphs	Abs_{t30}	0.324	0.232	13.750	(Vilcinskas *et al.*, 2013)
		Abs_{t20}	0.230	0.189	10.400	(Reeson *et al.*, 1998)
Highest value	• Sensitive to curves with a delayed increase • Sensitive to noise	Maximum rate of reaction within 30 minutes	0.015	0.012	0.871	(Rantala *et al.*, 2002)
Fixed Vmax	• Sensitive to curves with high absorbance at beginning and/or a delayed increase	Slope between 5 and 15 min	0.001	0.001	0.099	(Siva-Jothy *et al.*, 2008)

de famille – c'est-à-dire juste après l'éclosion des œufs. Nous avons ensuite transféré les familles dans un nouvel environnement standardisé (sans mère et sans pathogène) et suivi le développement de deux paramètres immunitaires chez les juvéniles au deuxième, troisième et quatrième stade de développement (les adultes ont aussi été testés mais les résultats ne sont pas présentés ici). Les paramètres immunitaires mesurés sont la concentration en hémocytes, qui sont des cellules immunitaires circulant dans l'hémolymphe et qui sont impliqués dans la reconnaissance et l'encapsulation des pathogènes, et dans l'activité totale de phénoloxidase (PO+PPO), qui est une cascade enzymatique principalement impliquée dans la mélanisation des corps étrangers (Beckage, 2008). Il est important de noter que pour mesurer l'activité totale de phenoloxidase, nous avons utilisé le script PO-CALC que nous avons précédemment développé afin de standardiser et d'optimiser la comparaison de ces mesures chez les invertébrés (Tableau 2 ; **Kohlmeier *et al.*, 2015**).

Nos résultats confirment que l'immunité des juvéniles augmente avec l'âge (Figure 22a ; Vogelweith *et al.*, 2017). Par contre, et de façon plus surprenante, nous avons montré que la présence de la mère et/ou des pathogènes dans les premiers jours post-éclosion n'influencent pas l'investissement des juvéniles dans leur concentration en hémocytes et dans leur activité totale de phenoloxidase (Figures 22b et 22c). Il n'y a pas non plus d'interaction entre les trois facteurs testés sur l'immunité des juvéniles.

Dans l'ensemble, ces résultats révèlent le rôle limité de la mère sur l'immunité personnelle des juvéniles, renforçant un peu plus l'idée selon laquelle mère et juvéniles sont très indépendants chez cette espèce. Il est toutefois important de noter que nous ne testons ici que l'effet à long terme de la présence d'une mère sur l'immunité des juvéniles, c'est-à-dire après que la mère ait cessé d'interagir avec ses juvéniles, ce qui n'exclut pas un effet de la mère sur l'immunité des juvéniles pendant la vie de famille. Pour autant, l'aide que la mère apporte à ses œufs (Boos *et al.*, 2014) et qu'elle pourrait potentiellement apporter à ses

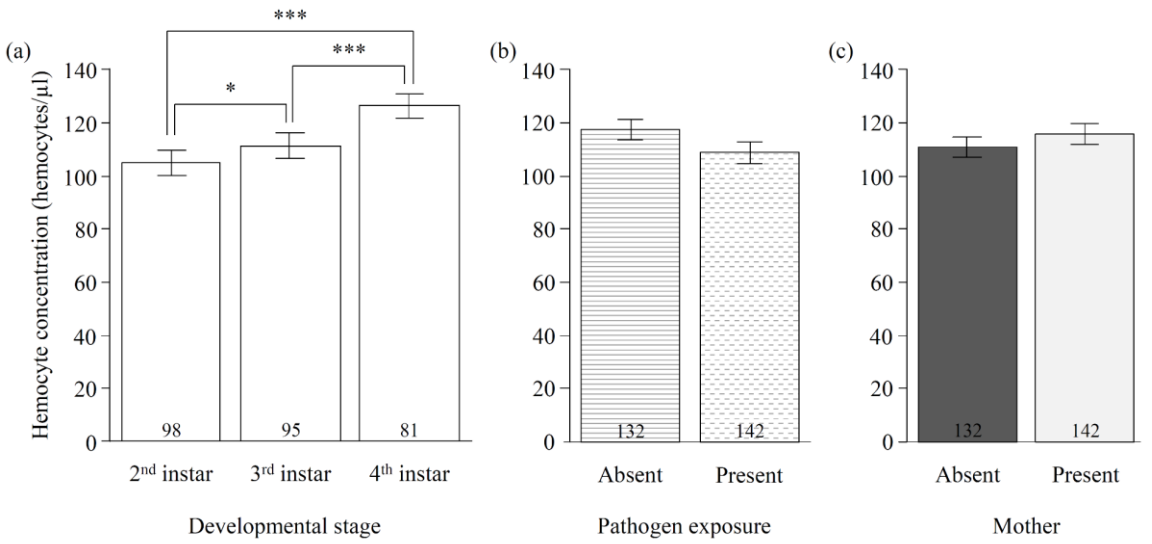

Figure 22 | Effets de (**a**) l'âge, (**b**) de la présence de pathogènes dans l'environnement et (**c**) d'une mère sur la concentration en hémocytes des descendants. Adapté de Vogelweith *et al.* (2017).

jeunes ne se retrouve pas une fois la vie de famille terminée. La protection contre les pathogènes ne semble donc pas être un moteur important dans le maintien des interactions mère-juvéniles chez cette espèce.

3.3.4 Les fécès peuvent-ils être une forme d'immunité sociale ?

- Diehl JM, Körner M, Pietsch M & **Meunier J** (2015). *BMC Evolutionary Biology, 15(1), 15:40.*

Est-ce que la vie de famille chez les forficules pourrait tout de même offrir une meilleure défense contre les pathogènes ? Chez les insectes sociaux, une forme d'immunité sociale particulièrement intéressante consiste à incorporer à l'intérieur du nid des matériaux aux propriétés antimicrobiennes. Un exemple classique se retrouve chez les fourmis des bois *Formica paralugubris.* Dans cette espèce, les ouvrières incorporent dans leur nid de grandes quantités de résine de conifère dont les propriétés antimicrobiennes permettent d'inhiber la croissance de microorganismes et servent donc à protéger la colonie contre de potentiels pathogènes (Christe *et al.*, 2003; Castella *et al.*, 2008). Ces matériaux ne sont pas toujours exogènes et peuvent être directement produits par les membres du groupe. C'est le cas du termite *Coptotermes formosanus* (Chouvenc *et al.*, 2013). Chez cette espèce, il a été montré que les fécès servaient de substrat pour le développement d'actinobactéries aux propriétés antifongiques. En maintenant leurs fécès à l'intérieur de la colonie, les ouvriers de cette espèce de termite empêchent donc l'installation de champignons entomopathogène dans leur proximité directe (Chouvenc *et al.*, 2013). Les propriétés antimicrobiennes des fécès ont aussi été démontré chez des espèces d'insectes non-eusociaux telles que le scarabée nécrophore *Nicrophorus vespilloides*. Chez cette espèce, les parents et leurs larves vivent sur des carcasses de vertébrés en décomposition sur lesquelles une grande concentration et diversité de microorganismes se développent (Rozen *et al.*, 2008). Les parents de cette espèce maintiennent donc leurs fécès aux propriétés antimicrobiennes sur les carcasses afin de lutter contre les microorganismes et ainsi monopolisent la ressource (Rozen *et al.*, 2008; Arce *et al.*, 2012) et empêchent l'exposition à de potentiels pathogènes (Reavey *et al.*, 2014; Duarte *et al.*, 2015).

Chez le forficule européen, nous avons observé que les juvéniles et la mère gardent leurs fécès à l'intérieur du nid (Diehl *et al.*, 2015). Nous nous sommes donc posés la question de savoir si ce phénomène pourrait aussi refléter une forme d'immunité sociale **(Diehl *et al.*, 2015**). Pour ce faire, nous avons collecté les fécès produits par les mères et les juvéniles de 17 familles. Avec ces fécès, nous avons ensuite réalisé plus de 400 tests d'inhibition de croissance contre les bactéries GRAM+ (*Bacillus subtilis* et *Staphylococcus aureus*) et GRAM- (*Escherichia coli*), un champignon (*Saccharomyces cerevisiae*) et d'une moisissure (*Aspergillus niger*). Nous avons aussi utilisé deux solutions contrôles (nourriture standard de forficule et solution tampon) afin de confirmer que nos résultats ne reflétaient pas un effet antimicrobien de la nourriture donnée aux forficules et/ou de la solution tampon dans laquelle les fécès ont été dilués. Nos résultats démontrent que les fécès des juvéniles inhibent la croissance des deux bactéries GRAM+, du champignon et de la moisissure, mais pas de la bactérie GRAM- et des deux contrôles (Figure 23). A l'échelle de la famille, cette activité est généralement plus forte dans les fèces de juvéniles (qui sont plus nombreux) que ceux des mères (Figure 23a),

même si l'activité antimicrobienne par mg de fécès est généralement plus haute chez les mères que chez les juvéniles (Figure 23b). Ces résultats révèlent donc que le maintien des fécès à l'intérieur du nid permet de limiter le développement de différents types de microbes et donc de protéger les membres de la famille contre de potentiels pathogènes. Ils soulèvent aussi deux questions. D'abord, pourquoi est-ce que l'activité antimicrobienne des fécès de la mère diffère de celle des juvéniles? Cette effet âge-spécifique pourrait refléter des différences (i) de composés résiduels de l'immunité personnelle (ces composés sont souvent présents dans les fécès des insectes, voir par exemple Shao *et al.*, 2012), (ii) de quantité de microorganismes intestinaux souvent impliqués dans la résistance aux infections (Kaltenpoth, 2009) ou encore (iii) de produits chimiques relâchés pendant la défécation entre les juvéniles

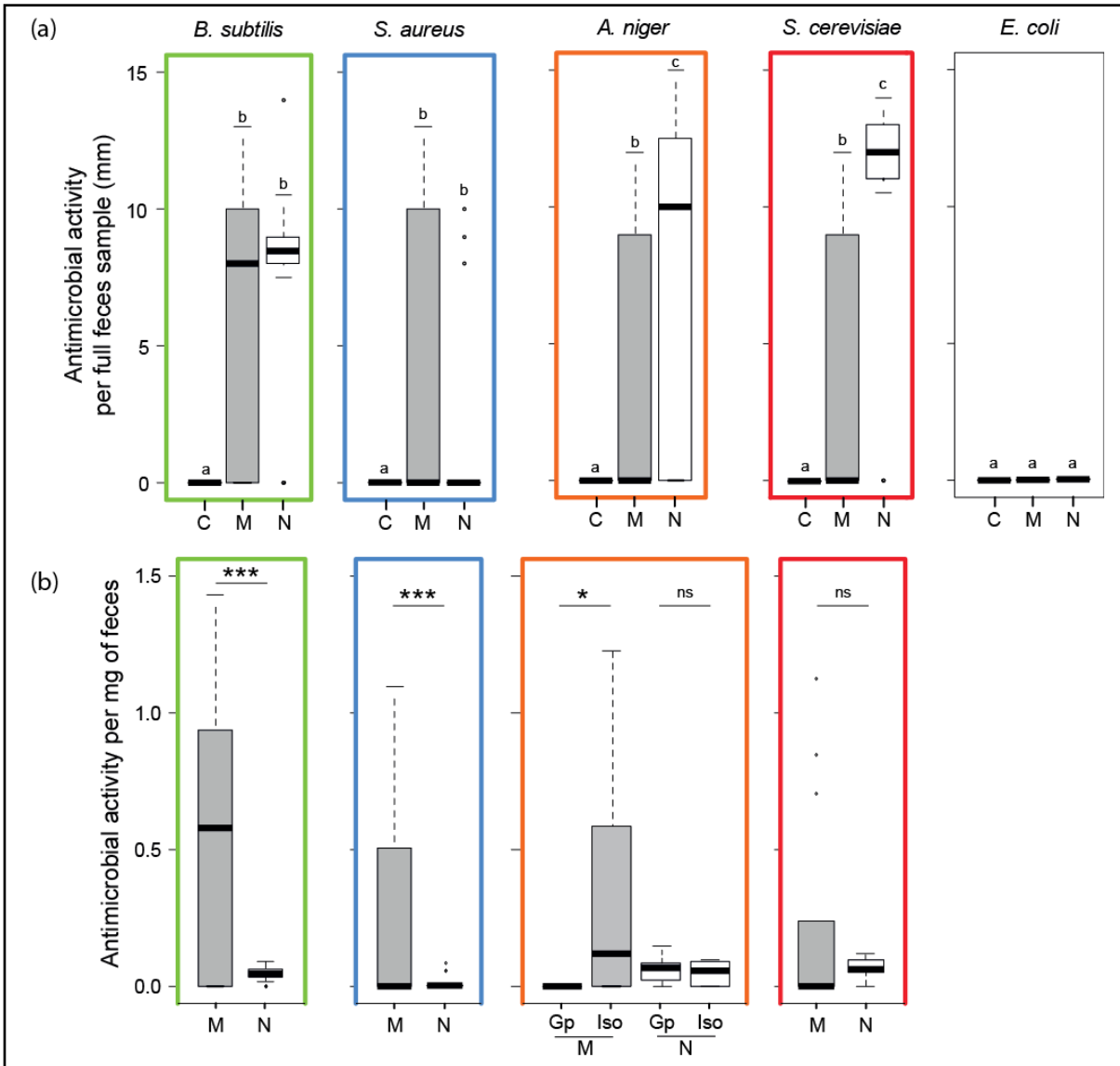

Figure 23 | Effets des fécès produits par la mère (M), les juvéniles (N) ou des solutions contrôles (C) sur la croissance de bactéries GRAM+ (*Bacillus subtilis* and *Staphylococcus aureus*) et GRAM- (*Escherichia coli*), d'un champignon (*Saccharomyces cerevisiae*) et d'une moisissure (*Aspergillus niger*). Les valeurs sont données soit (**a**) par quantité totale de fécès produits par le groupe concerné ou (**b**) par mg de fécès produits. Les mères et les juvéniles ont été maintenus ensemble ou séparés avant la collecte de fécès (Gp, Iso, résultat non commenté ici). Différentes lettres $p < 0.05$. Figure adaptée de Diehl *et al.* (2015).

et les adultes. De prochaines études devraient permettre de démêler ces hypothèses. La deuxième question concerne l'absence totale d'effet inhibiteur contre *E. coli*. De façon intéressante, il a été récemment démontré que la réponse immunitaire des insectes peut être assez spécifique en cas d'exposition à *E. coli,* et notamment que – contrairement à d'autres bactéries - cette exposition n'induit pas d'effet de priming immunitaire chez le scarabée *Tenebrio molitor* (Dubuffet *et al.*, 2015; Dhinaut *et al.*, 2017). Pour autant, il est important de souligner que suite à nos résultats (Diehl *et al.*, 2015), nous avons réalisé la même expérience avec une autre souche d'*E. coli* et que dans ce deuxième cas, les fécès avaient clairement un effet d'inhibiteur de croissance (Meyer Julia, Pietsch Michael et Meunier Joël, données non-publiées). Il semble donc que le système immunitaire des insectes réagisse de façon très spécifique à la souche d'*E. coli* utilisée pour le stimuler. Ces données suggèrent donc dans l'ensemble qu'il faut être prudent dans la généralisation des informations sur la résistance immunitaire des insectes contre les bactéries GRAM négatives lorsque l'on se base sur des résultats obtenus avec une seule souche d'*E. coli*.

3.3.5 Effet du groupe ou de l'isolement sur la protection contre les pathogènes ?

- Kohlmeier P, Holländer K & **Meunier J** (2016). *Journal of Evolutionary Biology*, 29, 1867–1872

Les études s'intéressant à la protection contre les pathogènes chez les insectes sociaux se sont souvent focalisées sur les effets de l'immunité sociale. Ainsi, il est généralement prédit que si les individus exposés à des pathogènes vivent plus longtemps lorsqu'ils sont maintenus en groupe plutôt que lorsqu'ils sont expérimentalement isolés, alors une immunité sociale est à l'œuvre (voir par exemple Hughes *et al.*, 2002). Pour être certain que cet effet ne reflète pas un stress létal due à une isolation sociale non-naturelle, ces études comparent aussi la survie des individus non-exposés à des pathogènes et maintenus en groupe ou en isolement. Mais ce contrôle oublie l'effet spécifique du stress d'un isolement social soudain sur la résistance aux pathogènes (plutôt que sur la survie générale des individus). Ce stress est pourtant connu chez de nombreuses espèces, chez qui il est responsable de changements profonds dans l'expression de traits comportementaux, physiologiques et neurologiques (Boulay *et al.*, 1999; Hawkley & Cacioppo, 2003; Hawkley & Capitanio, 2015; Koto *et al.*, 2015). Pour mettre en avant l'expression d'une immunité sociale, il semble donc primordial de vérifier qu'un soudain isolement social ne diminue pas la résistance immunitaire de chacun des individus.

Pour démontrer l'importance de ce stress sur la résistance aux pathogènes et plus généralement l'importance de contrôler cet effet lorsque l'on teste l'immunité sociale chez une espèce donnée, nous avons travaillé sur des groupes d'adultes du forficule européen **(Kohlmeier *et al.*, 2016**). En particulier, nous avons suivi la survie de mâles et de femelles pendant 25 jours après les avoir exposé à une solution de spores du champignon pathogène *M. brunneum* ou à une solution contrôle. Ces individus étaient répartis entre trois traitements : 1) ils étaient maintenus en groupe pendant 3 semaines, puis exposés à une des deux solutions et enfin remis dans le même groupe (= vie de groupe constante), 2) ils étaient maintenus en groupe pendant 3 semaines, puis exposés à une des deux solutions et enfin maintenus en isolement (= isolement social soudain) ou 3) ils étaient maintenus en isolement

pendant 3 semaines, puis exposés à une des deux solutions et enfin mis à nouveau en isolement (= isolement social constant). Comme nous l'avions prédit, la survie des femelles est plus faible dans le cas d'un isolement social soudain, que dans ceux d'un isolement social constant ou d'une vie de groupe constante (Figure 24). Par contre, et de façon surprenante, leur survie est la même lorsqu'elles sont constamment maintenues en groupe ou en isolation (Figure 24). L'isolement social soudain peut donc réduire à lui seul la résistance contre les pathogènes. En l'absence d'un tel contrôle expérimental, il aurait donc pu être possible de conclure à l'expression d'une immunité sociale dans les groupes de femelles du forficule européen, ce qui aurait été faux. De façon intéressante, chez les mâles, les résultats sont moins clairs et suggèrent que vivre en groupe avant l'exposition aux pathogènes engendre un coût immunitaire, mais que ce coût est moins apparent lorsque les mâles continuent de vivre en groupe après cette exposition (Figure 24). Ce phénomène pourrait s'expliquer par la grande agressivité des mâles envers les autres mâles chez cette espèce (Forslund, 2000), une agressivité qui est souvent connue pour être négativement associée au niveau de compétence immunitaire des insectes (Contreras-Garduño *et al.*, 2009; Adamo *et al.*, 2015).

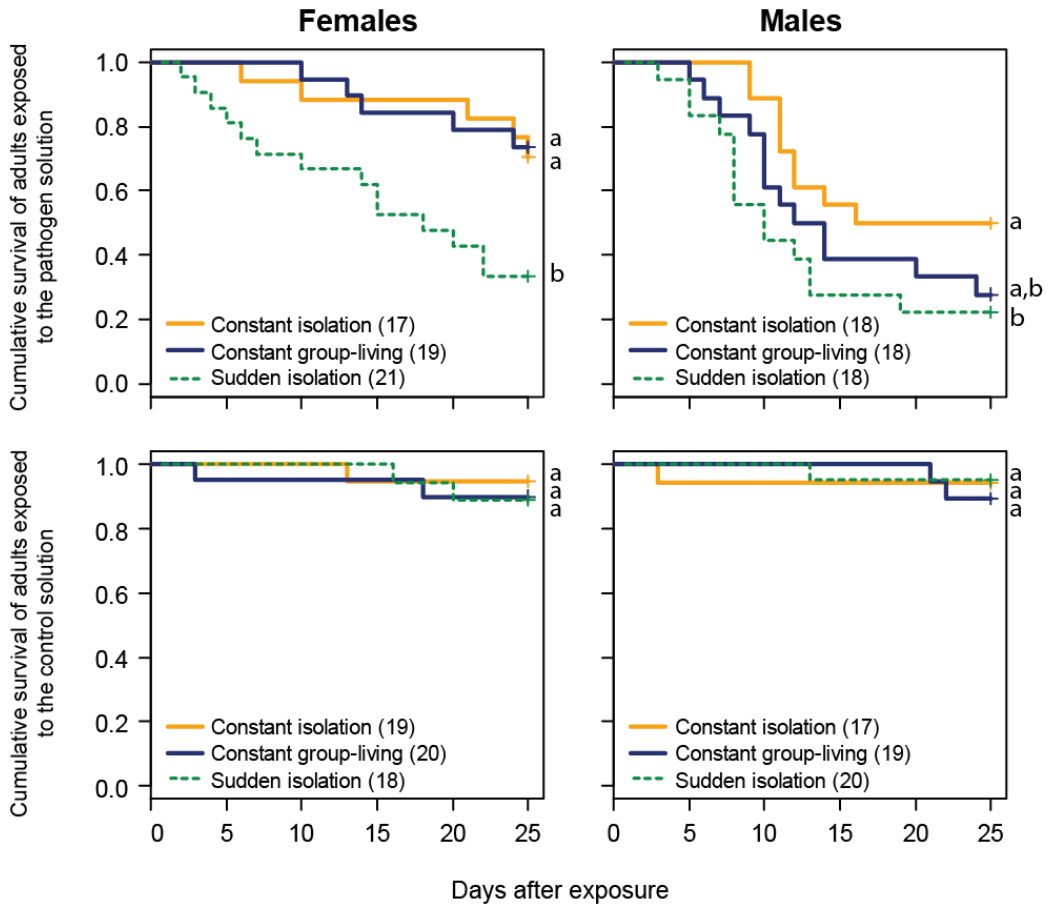

Figure 24 | Effets de l'exposition aux pathogènes et de l'isolement social sur la survie des adultes. Les groupes étaient toujours du même sexe que l'individu testé. Les tailles d'échantillons sont indiquées entre parenthèses. Différentes lettres $p < 0.05$. Figure tirée de Kohlmeier *et al.* (2016)

4. DISCUSSION ET PERSPECTIVES GENERALES

Mes travaux sur les fourmis et les forficules illustrent que la vie sociale chez les insectes repose sur un jeu complexe entre les conflits pour l'accès des individus à la reproduction (Meunier *et al.*, 2008, 2010) ou à une ressource limitante (Meunier & Kölliker, 2012a; b; Kölliker *et al.*, 2015), et les bénéfices d'une coopération visant le partage de la nourriture (Falk *et al.*, 2014; Kramer *et al.*, 2015) et la défense contre les pathogènes (Diehl *et al.*, 2015; Meunier, 2015). Ces résultats révèlent aussi qu'il est parfois difficile de prédire l'émergence d'un conflit ou d'une coopération, car ces deux processus peuvent impliquer les mêmes parties et faire intervenir les deux types d'action de façon quasi-simultanée. Par exemple, les juvéniles du forficule européen peuvent échanger de la nourriture mais aussi s'entre-tuer pour son accès (Dobler & Kölliker, 2010; Meunier & Kölliker, 2012b; Falk *et al.*, 2014; Kramer *et al.*, 2015), ou encore les mères forficules peuvent apporter de la nourriture à leurs juvéniles mais aussi entrer en compétition avec eux pour la monopoliser (Meunier & Kölliker, 2012a; b; Thesing *et al.*, 2015). Enfin, ces résultats soulèvent de nombreuses questions qu'il sera crucial d'étudier afin de mieux appréhender les mécanismes responsables de l'évolution de la vie sociale chez les insectes, et plus généralement ceux impliqués dans la transition évolutive entre vie solitaire et vie de groupe. Je souhaiterais terminer ce mémoire en détaillant cinq éléments qui me semblent particulièrement prometteurs et vers lesquels je souhaiterai diriger mes recherches dans les années à venir : (1) les bénéfices négligés des interactions familiales, (2) le rôle du microbiote intestinal dans l'évolution de la vie de groupe, (3) les implications des immunités sociale et personnelle dans l'évolution de la vie sociale, (4) les bases génétiques et épigénétiques de la vie familiale et enfin (5) l'utilisation des Dermaptères pour mieux comprendre la trajectoire évolutive de la subsocialité.

4.1 Les bénéfices négligés des interactions familiales

En révélant que les interactions entre juvéniles peuvent être associées à des bénéfices (Falk *et al.*, 2014; Diehl *et al.*, 2015; Kramer *et al.*, 2015; Körner *et al.*, 2016) et pas uniquement à des coûts (Mock & Parker, 1997)(voir aussi Roulin & Dreiss, 2012) au sein d'une famille, nos travaux appellent à élargir notre vision actuelle des interactions familiales et à approfondir notre connaissance sur les coûts et bénéfices qui leurs sont associés (Figure 25). Je propose ainsi de reconsidérer la nature des interactions entre parents et enfants. Des vertébrés aux invertébrés, il est généralement proposé que ces interactions apportent des bénéfices pour les enfants (du fait des soins parentaux) et des coûts pour les parents (Figure 25a ; Hinde *et al.*, 2010a; Klug & Bonsall, 2014). Nos travaux permettent de remettre en cause un des deux piliers de ce paradigme en démontrant que la présence d'une mère peut aussi induire des coûts pour les juvéniles, notamment lorsque la disponibilité en nourriture est faible (Meunier & Kölliker, 2012b; Thesing *et al.*, 2015; Kramer *et al.*, 2017). Qu'en est-il du second pilier, c'est-à-dire des coûts pour les parents ? Je propose ici que les interactions parents-enfants pourraient aussi être associées à des bénéfices directs pour les parents, c'est-à-dire des bénéfices autres que ceux visant à améliorer le développement et/ou la survie de leurs juvéniles jusqu'à l'âge adulte (Figure 25c ; Royle *et al.*, 2012). Les juvéniles pourraient en effet apporter de la nourriture à leurs parents (les juvéniles étant plus nombreux, leur efficacité de fourragement pourrait pallier à d'éventuelles déficiences chez leurs parents) ou les protéger

contre les parasites et pathogènes (par exemple, en retirant les pathogènes présents sur la surface des parents). Démontrer que de tels bénéfices existent dans les unités familiales facultative (c'est-à-dire une espèce précociale) aurait un impact majeur dans notre compréhension de l'évolution de la vie de famille et plus généralement de la vie de groupe chez les animaux. Ces bénéfices pourraient en effet expliquer - au moins en partie - pourquoi les parents restent avec leur juvéniles lorsque les bénéfices des soins parentaux ne sont pas requis pour ces derniers, un scénario qui reflète probablement les caractéristiques des juvéniles lorsque la vie de groupe a émergé à partir d'un état solitaire (Falk *et al.*, 2014). L'existence de ces bénéfices pourrait donc donner du poids à un scénario alternatif quant à l'émergence de la vie de famille dans la nature, en suggérant que la pression de sélection sur le maintien des parents avec les juvéniles ne repose pas uniquement sur les bénéfices liés au meilleur développement de leurs juvéniles (voir plus haut), mais sur les bénéfices directs qu'ils pourraient obtenir au détriment de leur propres juvéniles – en quelques sorte un scénario évolutif relevant du parasitisme social.

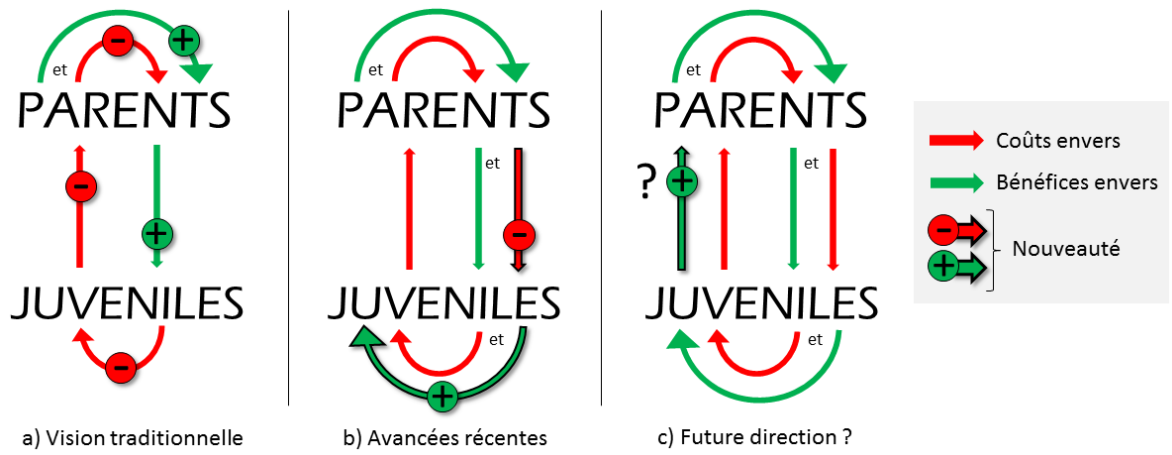

Figure 25 | Evolution de notre compréhension générale de la nature des interactions sociales exprimées au sein des familles animales.

4.2 Le microbiote intestinal et l'évolution de la socialité

Tous les organismes vivants ont un microbiote intestinal (c'est-à-dire des symbiotes et/ou une communauté microbienne résidant dans leur intestin) connu pour jouer un rôle essentiel dans l'expression de nombreux traits d'histoire de vie (Engel & Moran, 2013; Stilling et al., 2014). Ce microbiote est ainsi impliqué dans des comportements de reconnaissance entre individus chez les vertébrés et invertébrés (Sonnenburg, 2005; Lizé et al., 2007, 2013; Archie & Theis, 2011; Hongoh, 2011; Littman & Pamer, 2011; Rosengaus et al., 2011; Ezenwa et al., 2012; Archie & Tung, 2015; Flórez et al., 2015), dans des maladies humaines telles que la dépression, l'anxiété et l'autisme (Sekirov et al., 2010; Grenham et al., 2011; Stilling et al., 2014) ou encore dans des processus physiologiques influençant la réponse immunitaire ou la croissance des juvéniles (Ponton et al., 2011; Chambers & Schneider, 2012; Coon et al., 2014). Au cours de la dernière décennie, une hypothèse importante s'est développée selon laquelle le

microbiote intestinal pourrait être un moteur évolutif central de la vie sociale (Troyer, 1984; Nalepa et al., 2001; Lombardo, 2008; Lewin-Epstein et al., 2017). Cette hypothèse repose sur le fait que, pour favoriser sa transmission entre hôtes, le microbiote intestinal devrait favoriser le rapprochement géographique de ces hôtes, la fréquence de leurs contacts et l'expression de comportements coopératifs tels que la trophallaxie et/ou la coprophagie. Cette hypothèse est étayée par de nombreuses études chez les insectes eusociaux (termites et abeilles, principalement), chez qui les comportements sociaux sont connus pour permettre le transfert du microbiote intestinal (ou du moins une partie) entre les membres de la colonie (Nalepa, 1994; Powell et al., 2014). En comparaison, le lien entre microbiote et interactions sociales est beaucoup moins étudié chez les espèces non-eusociales, et plus particulièrement chez celles avec une vie de famille. Chez ces dernières, les quelques études disponibles s'intéressent principalement au transfert vertical du microbiote entre la mère et les juvéniles (voir par exemple chez les humain, Kuang et al., 2016). Mieux comprendre le rôle du microbiote intestinal dans l'émergence et le maintien de la vie sociale à partir d'un état solitaire pourrait donc se faire en étudiant la nature et l'importance de ce lien chez les espèces formant des groupes non-eusociaux telles que le forficule européen. Nos résultats suggèrent déjà que le microbiote intestinal pourrait jouer un rôle important chez cette espèce. Ils montrent en effet que les juvéniles échangent activement des substances initialement présentes dans leur système digestif par trophallaxie proctodéale (bouche-anus) et par coprophagie (consommation de fécès), mais aussi que les contacts bouche-bouche sont fréquents (Falk et al., 2014). Ils montrent de plus que même si ces échanges permettent d'augmenter la survie des juvéniles en l'absence de nourriture, ils sont indépendants de leur besoin nutritionnel et qu'ils restent importants lorsqu'une source de nourriture est présente à proximité (Falk et al., 2014; Körner et al., 2016). Le rôle de ce transfert ne semble donc pas limité à l'échange de matériels nutritifs et une hypothèse probable est qu'il permette aussi l'échange du microbiote intestinal. Mes prochains projets de recherche vont approfondir ces résultats afin de décrire la nature du microbiote intestinal chez *F. auricularia* et de mieux comprendre son importance dans les traits d'histoire de vie de cette espèce. Au travers d'expériences où l'on manipulera la présence et/ou la composition de ce microbiote, nous allons notamment tester son rôle dans l'expression des comportements familiaux (dont les soins maternels), dans la résistance contre les pathogènes et plus généralement son implication dans le maintien de la vie de famille.

4.3 Immunité sociale et immunité personnelle

Comme énoncé précédemment, la vie de groupe augmente les risques qu'un individu soit exposé et infecté par des pathogènes (voir par exemple Ugelvig & Cremer, 2007; Rifkin et al., 2012; Stroeymeyt et al., 2014). A ce jour, les études sur ce phénomène s'intéressent le plus souvent aux défenses collectives chez les insectes eusociaux (Schmid-Hempel, 1998; Cremer et al., 2007; Wilson-Rich et al., 2009b), laissant en suspens des questions importantes liées à son fonctionnement global et à son rôle dans l'évolution de la vie sociale (Meunier, 2015). Il est par exemple encore difficile de comprendre si l'immunité sociale est un processus ancestral qui a permis aux groupes de se structurer et d'augmenter en complexité organisationnelle, ou s'il s'agit d'un processus secondaire qui a émergé après l'apparition des

groupes complexes pour assurer leur fonctionnement et leur maintien malgré leur fixation (c'est-à-dire l'obligation de la vie de groupe) et la pression parasitaire importante. Démêler ces scenarii évolutifs demande d'étudier la présence et la nature de l'immunité sociale chez les espèces non-eusociales chez qui les individus peuvent à la fois vivre en groupe et de façon solitaire (Meunier, 2015). Avec les scarabées nécrophores (par exemple Reavey et al., 2014a; b; Duarte et al., 2015; Palmer et al., 2016), le forficule européen constitue un modèle biologique de plus en plus utilisé pour ce genre d'étude. De par sa biologie unique, il est en effet possible d'y manipuler « naturellement » la présence d'un environnement social et donc de tester l'importance des pathogènes sur l'émergence et le maintien de sa vie de groupe. Nos premiers résultats chez le forficule européen suggèrent que le rôle positif des interactions familiales dans la défense contre les pathogènes ne vient pas nécessairement des soins maternels et qu'il peut se reposer sur d'autres mécanismes liés aux stress de l'isolement social (voir par exemple Diehl et al., 2015; Kohlmeier et al., 2016; Vogelweith et al., 2017). Nos travaux à venir viseront à confirmer ou infirmer ces conclusions, mais aussi à explorer plus en détail l'importance du groupe sur la résistance contre les pathogènes chez cette espèce. Ces études devraient nous permettre d'avoir un éclairage nouveau dans la chronologie évolutive du lien entre évolution sociale et résistance contre les pathogènes.

Mieux comprendre le rôle des pathogènes dans l'évolution de la vie sociale demande aussi de s'intéresser à l'immunité personnelle des individus. L'immunité personnelle est un processus bien connu chez les vertébrés et les invertébrés qui implique à la fois des défenses physiologiques et comportementales de la part de l'individu infecté (Beckage, 2008; de Roode & Lefèvre, 2012; Masri & Cremer, 2014). Ces défenses englobent, par exemple, des réactions enzymatiques particulières cherchant à isoler et à éliminer les pathogènes présents dans le sang/l'hémolymphe, ou encore la collecte et/ou la consommation de composés organiques visant à stopper une infection ou à réduire ses symptômes via automédication (de Roode et al., 2013). Investir dans l'immunité personnelle est coûteux, de sorte que les individus doivent optimiser cet investissement en fonction, par exemple, du risque d'infection, de leur propre qualité ou de l'importance d'investir dans d'autres traits d'histoire de vie. Parce qu'investir dans deux processus ayant la même fonction serait inutilement coûteux pour un individu, plusieurs études ont proposé d'explorer le compromis entre immunité personnelle et immunité sociale (Cremer *et al.*, 2007; Cotter & Kilner, 2010; Meunier, 2015). Des approches de génomique comparative ont récemment été réalisées dans ce but, proposant spécifiquement de tester la prédiction selon laquelle un accès à l'immunité sociale permettrait de réduire l'investissement dans l'immunité personnelle chez les insectes. A l'encontre de cette prédiction, les résultats de ces études démontrent que ce lien n'existe pas, en tout cas pas en ce qui concerne le nombre de gènes de l'immunité exprimés entre les espèces eusociales et non-eusociales (voir la revue par Otani et al., 2016). Les approches plus directes sont beaucoup moins nombreuses avec, à ma connaissance, une seule étude visant à tester expérimentalement ce compromis. Dans cette étude réalisée sur le scarabée nécrophore, Cotter *et al.* (2010) ont montré qu'augmenter son investissement dans la réparation d'une blessure (immunité personnelle) impliquait une diminution temporaire de l'investissement dans l'activité antimicrobienne des fécès (considéré ici comme un investissement dans l'immunité sociale) chez les mères. De prochaines études vont devoir

explorer ce lien entre les deux formes d'immunité, mais aussi les coûts physiologiques directs qui sont associés à l'immunité sociale. Le forficule européen sera un modèle d'étude particulièrement intéressant dans ce cadre.

4.4 Une approche génomique

Au cours de la dernière décennie, l'incursion de la génomique dans le domaine de l'écologie comportementale à entrainer le développement de nombreux axes de recherche. Elle a par exemple permis de rechercher les gènes et/ou réseaux de gènes impliqués dans de nombreux comportements (Fitzpatrick et al., 2005), d'étudier comment ces gènes/réseaux évoluent au cours du temps (Linksvayer et al., 2012) et de mettre en lumière les mécanismes moléculaires (par exemple épigénétiques) responsables de la plasticité comportementale d'un individu (Danchin et al., 2011; Patalano et al., 2012). Elle a aussi permis de manipuler l'expression d'un comportement (par exemple par l'injection d'ARNi) afin d'en mesurer les effets directs et indirects sur la fitness de l'individu testé (Liang et al., 2012), ou encore de mieux comprendre les contraintes moléculaires favorisant l'évolution convergente ou divergente de comportements à travers les espèces animales (Robinson, 1999; Rittschof & Robinson, 2014) ou de prédire la transmission de la plasticité comportementale entre générations (Francis et al., 2003; Danchin et al., 2011).

Dans le cadre des comportements sociaux, la génomique (appelée alors sociogénomique) s'est jusqu'à aujourd'hui principalement intéressée aux traits phénotypiques inhérents au système eusocial, tels que la différentiation des castes et la division du travail (Amdam et al., 2004; Robinson et al., 2005; Abbot et al., 2011; Feldmeyer et al., 2014; Terrapon et al., 2014; Elsik et al., 2016). Outre les comparaisons d'expression génique entre espèces sœurs montrant des systèmes eusociaux et non-eusociaux (par exemple Pellens et al., 2007), assez peu d'informations existent sur la sociogénomique des interactions sociales dans les espèces d'insectes non-eusociales (chez les souris, voir Bendesky *et al.*, 2017). Chez les insectes, ces rares informations proviennent essentiellement d'études sur le scarabée nécrophore *N. Vespilloides* (Cunningham *et al.*, 2015; Benowitz *et al.*, 2017), une espèce d'insecte chez qui les femelles et les mâles s'occupent de leurs larves pendant les quelques jours suivant l'éclosion des œufs (Capodeanu-Nägler et al., 2016). Une de ces études a par exemple montré que le récepteur du gène neuropeptide F - qui est impliqué dans le fourragement des femelles - est moins exprimé pendant la phase de soins maternels, suggérant que l'évolution des soins parentaux repose aussi sur des changements d'expression des gènes associés à la prise de nourriture (Cunningham et al., 2016). Une autre étude a révélé que l'expression de la vitellogenine, une molécule fortement associée à la division du travail et à la spécialisation comportementale chez les abeilles (Amdam et al., 2003, 2004; Nelson et al., 2007; Amdam & Page, 2010), est aussi associée aux soins parentaux chez les mâles et les femelles de nécrophore. En particulier, l'expression de ce gène et de son récepteur est réduite dans le cerveau des parents lorsque ces derniers prodiguent des soins à leurs larves (Roy-Zokan et al., 2015). Enfin, la même équipe de chercheurs a démontré que la vie de famille était associée à une expression particulière d'un ensemble de gènes, mais que la présence d'un seul ou des deux parents n'avait pas d'effet sur cette expression (Parker et al., 2015).

Ces premiers résultats sur les nécrophores sont particulièrement prometteurs, car ils permettent de mettre en avant les bases génétiques de la vie de famille et contribuent ainsi à notre compréhension des facteurs liés à son origine évolutive. Mais d'autres études sur d'autres modèles sont aujourd'hui nécessaires afin de déterminer l'universalité des résultats présentés, et plus largement leur importance dans l'évolution de la vie de groupe chez plusieurs espèces. La biologie particulière du forficule européen en fait un candidat idéal pour explorer les bases génétiques de la vie de famille et de la subsocialité chez les insectes. Les quelques travaux qui ont récemment étudié le transcriptome de cet insecte (Simon et al., 2012; Roulin et al., 2014) montrent que ce type d'analyse est réalisable et appellent à de nouveaux travaux visant à caractériser, par exemple, les gènes impliqués dans les comportements de soins parentaux (de la part des donneurs et des receveurs) et dans le maintien (ou non) de la vie de groupe chez les juvéniles.

4.6 L'évolution de la vie sociale chez les Dermaptères

L'ensemble de mes travaux sur le forficule européen *F. auricularia* a permis d'initier une réflexion globale sur la nature possible des transitions évolutives entre vie solitaire et vie sociale chez cette espèce. En particulier, l'apparente indépendance des juvéniles vis-à-vis des soins maternels, conjuguée aux coûts potentiels de la présence d'une mère sur la survie de ces juvéniles soulèvent la question de la direction évolutive de la vie de famille chez cette espèce : sommes-nous face à une espèce chez qui la vie de famille est en train de se complexifier, en train de se perdre ou bien chez qui elle représente un état évolutivement stable ? Répondre à cette question demande d'élargir nos connaissances sur les formes de vie sociale présentes chez les Dermaptères (Figure 26). Alors que cet ordre d'insectes compte

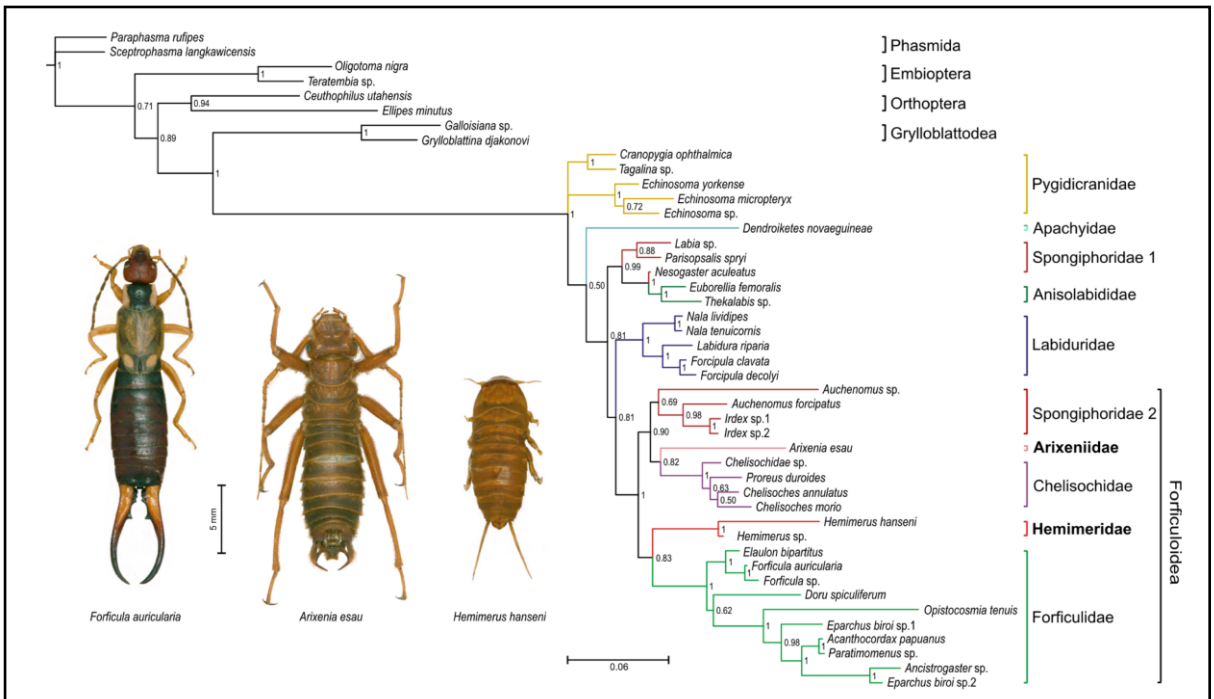

Figure 26 | Phylogénie des forficules. Basée sur une analyse bayésienne de séquences nucléaires. Figure tirée de Kocarek *et al.* (2013).

environ 2000 espèces principalement présentes dans les zones tropicales et subtropicales (Albouy & Caussanel, 1990)(Figure 22), la biologie de la très grande majorité des Dermaptères reste inconnue à ce jour. Outre les quelques espèces commensales de chauve-souris en Asie du Sud-est (famille Arixeniidae) ou semi-parasites de rongeurs en Afrique (famille Hemimeridae) et qui ont donc un mode de vie très particulier (Gullan & Cranston, 2005), les espèces de Dermaptères semblent présenter une grande diversité de formes subsociales. La littérature présente en effet quelques informations montrant que les soins aux œufs et/ou aux juvéniles ne sont pas présents chez toutes les espèces (Tableau 3). Pour autant, la très grande majorité de ces informations sont parcellaires et la répartition phylogénétique de ces formes sociales chez ces Dermaptères reste inexplorée. Les connaissances sur le forficule européen que j'ai pu développer au cours de ces dernières années devraient me permettre de collecter (au moins une partie) de ces informations manquantes et ainsi d'explorer plus en détail l'évolution de la vie de famille chez les Dermaptères.

Tableau 3 | Soins aux œufs et soins aux juvéniles chez plusieurs espèces de Dermaptères. Ces informations (encore préliminaires) ont été en partie collectées par Beatrice Dewienter lors d'un travail bibliographique que j'ai encadré en 2014. + présence, - absence, ?? information non-disponible.

Espèce	Famille	Avant éclosion		Après éclosion		N études*
		Soins aux oeufs	Nettoyage des oeufs	Vie de famille	Soins maternels	
Diplatys flavicollis	Diplatyidae	+	-	+	+	1
Tagakin papua	Pygidicranidae	+	-	+	+	1
Paracranopygia siamensis	Pygidicranidae	+	??	+	??	1
Anisolabis littorea	Anisolabididae	+	??	+	+	3
Anisolabis maritima	Anisolabididae	+	+	+	+	13
Euborellia annulipes	Anisolabididae	+	+	+	+	38
Euborellia cincticollis	Anisolabididae	+	+	+	+	1
Euborellia plebeja	Anisolabididae	+	??	??	??	1
Labidura riparia	Labiduridae	+	+	+	+	115
Nala lividipes	Labiduridae	+	+	??	??	10
Anechura harmandi	Forficulidae	+	+	??	??	7
Apterygida media	Forficulidae	+	??	??	??	3
Doru lineare	Forficulidae	+	+	+	+	1
Doru taeniatum	Forficulidae	+	+	+	+	10
Forficula auricularia	Forficulidae	+	+	+	+	197
Forficula lesnei	Forficulidae	+	+	+	+	1
Forficula senegalensis	Forficulidae	+	??	+	+	4
Labia minor	Spongiphoridae	+	+	+	+	1
Marava arachids	Spongiphoridae	-	-	-	-	5
Hamaxas nigrorufus	Spongiphoridae	+	??	??	??	1
Chaetospania borneensis	Spongiphoridae	-	-	-	-	1

* Nombre d'études faisant référence à cette espèce dans ISI web of Science en 2014.

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III. ANNEXES

SÉLECTION DE PUBLICATIONS

Liste des 21 publications décrites dans le texte et proposées en annexe.
Ces publications sont présentées suivant leur ordre d'apparition dans le mémoire.

(1) **Meunier J**, Delaplace L & Chapuisat M. 2010. Reproductive conflicts and egg discrimination in a socially polymorphic ant. *Behav. Ecol. Soc.* **64**: 1655–1663.

(2) **Meunier J**, West S A & Chapuisat M. 2008. Split sex ratios in the social Hymenoptera: a meta-analysis. *Behav. Ecol.* **19**: 382–390.

(3) **Meunier J** & Kölliker M. 2012. When it is costly to have a caring mother: food limitation erases the benefits of parental care in earwigs. *Biol. Lett.* **8**: 547–550.

(4) Wong J W Y*, **Meunier J***, Lucas C & Kölliker M. 2014. Paternal signature in kin recognition cues of a social insect: concealed in juveniles, revealed in adults. *Proc. R. Soc. B Biol. Sci.* **281**: 20141236. [*Authors contributed equally to the work]

(5) Kölliker M, Boos S, Wong J W Y, Röllin L, Stucki D, Raveh S, Wu M & **Meunier J**. 2015. Parent-offspring conflict and the genetic trade-offs shaping parental investment. *Nat. Commun.* **6**: 6850.

(6) **Meunier J** & Kölliker M. 2012. Parental antagonism and parent-offspring co-adaptation interact to shape family life. *Proc. R. Soc. B Biol. Sci.* **279**: 3981–8.

(7) Wong J W Y, **Meunier J** & Kölliker M. 2013. The evolution of parental care in insects: the roles of ecology, life history and the social environment. *Ecol. Entomol.* **38**: 123–137.

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(9) Thesing J, Kramer J, Koch L K & **Meunier J**. 2015. Short-term benefits, but transgenerational costs of maternal loss in an insect with facultative maternal care. *Proc. R. Soc. B Biol. Sci.* **282**: 20151617.

(10) **Meunier J** & Kölliker M. 2013. Inbreeding depression in an insect with maternal care: influences of family interactions, life stage and offspring sex. *J. Evol. Biol.* **26**: 2209–20.

(11) Kramer J, Körner M, Diehl J M C, Scheiner C, Yüksel-Dadak A, Christl T, Kohlmeier P & **Meunier J**. 2017 When earwig mothers do not care to share: parent-offspring competition and the evolution of family life. *Func. Ecol.* in press.

(12) Falk J, Wong J W Y, Kölliker M & **Meunier J**. 2014. Sibling cooperation in earwig families provides insights into the early evolution of social life. *Am. Nat.* **183**: 547–557.

(13) Kramer J, Thesing J & **Meunier J.** 2015. Negative association between parental care and sibling cooperation in earwigs: a new perspective on the early evolution of family life? *J. Evol. Biol.* **28**: 1299–1308.

(14) Kramer J & **Meunier J.** 2016. Maternal condition determines offspring behavior toward family members in the European earwig. *Behav. Ecol.* **27**: 494–500.

(15) Körner M, Diehl J M & **Meunier J**. 2016. Growing up with feces: benefits of allo-coprophagy in families of the European earwig. *Behav. Ecol.* **27**: 1775–1781.

(16) **Meunier J**. 2015. Social immunity and the evolution of group living in insects. *Philos. Trans. R. Soc. B Biol. Sci.* **370**: 20140102.

(17) Boos S, **Meunier J**, Pichon S & Kölliker M. 2014. Maternal care provides antifungal protection to eggs in the European earwig. *Behav. Ecol.* **25**: 754–761.

(18) Vogelweith F*, Körner M*, Foitzik S & **Meunier J**. (2017). Age, pathogen exposure, but not maternal care shape offspring immunity in an insect with facultative family life. *BMC Evol. Biol*, 17:69. [*Authors contributed equally to the work]

(19) Kohlmeier P, Dreyer H & **Meunier J.** 2015. PO-CALC: A novel tool to correct common inconsistencies in the measurement of phenoloxidase activity. *J. Insect Physiol.* **75**: 80–84.

(20) Diehl J M, Körner M, Pietsch M & **Meunier J**. 2015. Feces production as a form of social immunity in an insect with facultative maternal care. *BMC Evol. Biol.* **15**: 15:40.

(21) Kohlmeier P, Holländer K & **Meunier J.** 2016. Survival after pathogen exposure in group-living insects: don't forget the stress of social isolation! *J. Evol. Biol.* **29**: 1867–1872.

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ORIGINAL PAPER

Reproductive conflicts and egg discrimination in a socially polymorphic ant

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Abstract The ability to discriminate against competitors shapes cooperation and conflicts in all forms of social life. In insect societies, workers may detect and destroy eggs laid by other workers or by foreign queens, which can contribute to regulate reproductive conflicts among workers and queens. Variation in colony kin structure affects the magnitude of these conflicts and the diversity of cues used for discrimination, but the impact of the number of queens per colony on the ability of workers to discriminate between eggs of diverse origin has so far not been investigated. Here, we examined whether workers from the socially polymorphic ant *Formica selysi* distinguished eggs laid by nestmate workers from eggs laid by nestmate queens, as well as eggs laid by foreign queens from eggs laid by nestmate queens. Workers from single- and multiple-queen colonies discriminated worker-laid from queen-laid eggs, and eliminated the former. This suggests that workers collectively police each other in order to limit the colony-level costs of worker reproduction and not because of relatedness differences towards queens' and workers' sons. Workers from single-queen colonies discriminated eggs laid by foreign queens of the same social structure from eggs laid by nestmate queens. In contrast, workers from multiple-queen colonies did not make this distinction, possibly because cues on workers or eggs are more diverse. Overall, these data indicate that the ability of *F. selysi* workers to discriminate eggs is sufficient to restrain worker reproduction but does not permit discrimination between matrilines in multiple-queen colonies.

Keywords Worker policing · Nestmate recognition · Social insect · Ants · Hymenoptera · *Formica selysi*

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Introduction

The evolution of integrated societies requires processes moderating within-group conflicts and preventing group-exploitation by foreigners (Szathmary and Maynard Smith 1995; Keller and Chapuisat 1999; Michod and Roze 2001; Ratnieks et al. 2006). In insect societies, egg discrimination by workers may play an important role in both contexts. First, workers may distinguish and eliminate eggs laid by nestmate workers, and by so doing enforce their collective interest (leaving male production to the queens) over their individual interests (producing sons (Ratnieks 1988; Frank 1995; Wenseleers and Ratnieks 2006)). Second, workers may discriminate against eggs laid by foreign queens to prevent the exploitation and progressive invasion of colonies by unrelated conspecifics (Hamilton 1964; Crozier and Pamilo 1996).

Workers from many Hymenopteran societies (ants, bees, and wasps) are known to police the reproduction of nestmate workers. They either eliminate worker-laid eggs

that would otherwise develop into males or behave aggressively towards workers with developed ovaries (Ratnieks 1988; reviewed in Wenseleers and Ratnieks 2006; Helanterä and Sundström 2007b; van Zweden et al. 2007). Two major factors may promote worker policing. First, workers should police each other when they are on average more related to queen-produced males than to worker-produced males (the relatedness asymmetry hypothesis). Second, workers should prevent worker reproduction if it decreases colony productivity (the colony-level efficiency hypothesis). Both hypotheses are based on kin selection: policing evolves when workers increase their inclusive fitness by preventing the reproduction of other workers. However, the respective importance of each hypothesis remains a matter of debate (Hammond and Keller 2004; Wenseleers and Ratnieks 2006).

According to the relatedness asymmetry hypothesis, worker policing should depend on the social structure of the colony. Workers should police each other in colonies headed by one queen that had mated with more than two males, or in colonies headed by many queens of moderate to high relatedness (Ratnieks 1988; Crozier and Pamilo 1996). A comparative analysis provided partial support to the relatedness asymmetry hypothesis: the 75 species of social Hymenoptera wherein workers were most closely related to worker-produced males had a greater proportion of males produced by workers (14%) than the 15 species wherein workers were most closely related to queen-produced males (which had less than 2% of worker-produced males); (e.g., Foster et al. 2002; Iwanishi et al. 2003; reviewed Wenseleers and Ratnieks 2006). However, policing is also common in species with one singly mated queen, in which workers are more related to the sons of other workers than to the sons of the queens (e.g., Foster et al. 2002; Iwanishi et al. 2003; reviewed Wenseleers and Ratnieks 2006). This suggests that relatedness asymmetry is not the only factor selecting for worker policing.

According to the efficiency hypothesis, worker policing evolves because the colony-level costs of worker reproduction exceed the inclusive fitness gains of rearing workers' sons instead of queens' sons (Ratnieks 1988; Hammond and Keller 2004; Wenseleers and Ratnieks 2006). Worker reproduction can decrease colony efficiency, and hence colony productivity, if reproducing workers work less (Cole 1986; Gobin et al. 2003), or if competition among reproducing workers leads to an over-production of eggs that exceeds the rearing capacity of the colony (Hardin 1968; Wenseleers et al. 2004). Despite these potential costs, workers rear a large proportion of workers' sons in some species (reviewed in Hammond and Keller 2004; Wenseleers and Ratnieks 2006), which suggests that the costs and benefits of worker reproduction vary among species and depend on colony life history (e.g., Dijkstra and Boomsma 2007).

The ability of workers to discriminate nestmate from foreign eggs is important to maintain colony integrity. In various social insect species, conspecific queens occasionally infiltrate established colonies (Beekman and Oldroyd 2008; Holzer et al. 2008a). This dilutes the relatedness among nestmates and decreases the inclusive fitness of resident workers (Hamilton 1964; Crozier and Pamilo 1996). Inter-specific social parasitism is also frequent in ants, with parasitic queens taking over the nests of their host species to lay their own brood (e.g., Kutter 1977). Hence, resident workers should endeavor to eliminate non-nestmate queens and their brood, whereas the foreign queens and brood should try to escape detection.

Workers from numerous ant species aggressively reject foreign queens seeking adoption in their colonies (e.g., Sundström 1997; Kikuchi et al. 2007; but see Vasquez and Silverman 2008; Holzer et al. 2008b). In contrast, workers frequently do not discriminate against foreign queen-laid eggs (e.g., Ratnieks and Visscher 1989; Foster and Ratnieks 2001; Martin et al. 2002; Pirk et al. 2003; Endler et al. 2004). Proximately, it has been suggested that this absence of discrimination was due to an "acceptance" pheromone signal displayed on queen-laid eggs and conserved across colonies (Vander Meer et al. 1998). Ultimately, egg discrimination may be selected against because discrimination errors or nepotistic behavior generated by competition among matrilines within colonies are costly (Keller 1997; Holzer et al. 2006). Recent studies did however find evidence that workers were able to discriminate against eggs laid by foreign queens in the ant *Formica fusca* (Helanterä and Sundström 2007a) and the honeybee *Apis mellifera* (Pirk et al. 2007). These new results call for further studies on the ability of workers to discriminate against foreign eggs, as well as on the factors modulating workers' response towards nestmate and foreign queen-laid eggs.

Variation in colony kin structure might affect nestmate discrimination by modifying the degree of relatedness within colonies and the diversity of cues used by workers to recognize nestmate from foreigners. To recognize intruders, social insects compare their chemical profile to a learned chemical template that characterizes each colony (Vander Meer and Morel 1998). Colonies with multiple queens contain a broader mix of chemical cues, thus increasing discrimination errors (Hölldobler and Wilson 1977; Breed and Bennett 1987; Vander Meer and Morel 1998) and possibly leading to a higher degree of tolerance towards conspecific foreigners (reviewed in Bourke and Franks 1995; Sundström 1997; but see Rosset et al. 2006). So far, the influence of the number of queens per colony on the ability of workers to discriminate against foreign eggs has not been investigated.

The aim of this study was to investigate the influence of social structure variation on the ability of workers to discriminate against eggs from various origins. Our model system was the socially polymorphic ant *Formica selysi*, in which single-queen (monogyne) and multiple-queen (polygyne) colonies coexist in the same population (Chapuisat et al. 2004). There was no sign of genetic differentiation between social forms at microsatellite markers (Chapuisat et al. 2004). Gene flow between social forms may occur because queens originating from both monogyne and polygyne colonies can successfully mate with males from the alternative social form and seem able to found colonies independently (Reber et al. 2010). In our study population, we detected little change in the social structure of colonies when genotyping the same colonies over several years (Chapuisat et al. 2004; Schwander et al. 2005; Rosset et al. 2006; Reber et al. 2008; Meunier and Chapuisat 2009). This suggests that shifts in social structure are rare and that the social conditions may be stable enough to permit the evolution of adaptive brood discrimination by workers depending on the social structure of the colony, provided that the selection pressure is high enough (see also Rosset and Chapuisat 2007; Meunier and Chapuisat 2009 for further differences between social forms).

We used egg-acceptance bioassays to examine whether workers from each type of social background discriminated (1) eggs laid by nestmate workers from eggs laid by nestmate queens, as expected if worker policing occurs and (2) eggs laid by foreign queens from eggs laid by nestmate queens, as expected if nestmate discrimination of eggs occurs. In colonies of our study population, the actual degree of relatedness asymmetry should not promote worker policing (see "Material and methods" section for estimates of relatedness asymmetry). Workers from monogyne colonies are more related to workers' sons than to queens' sons, whereas workers from polygyne colonies are equally related to both types of males. Hence, the relatedness asymmetry hypothesis predicts that workers should not discriminate against eggs laid by nestmate workers, particularly in monogyne colonies. In contrast, the selective elimination of worker-laid eggs in monogyne colonies, or in both types of colonies, would support the efficiency hypothesis (Hammond and Keller 2004; Wenseleers and Ratnieks 2006).

We also predict that workers from polygyne colonies might be more tolerant of eggs laid by foreign queens than workers from monogyne colonies. This prediction could result from either of the two factors: polygyne colonies could contain more diverse recognition cues preventing accurate nestmate discrimination. Alternatively, the cost of intra-colony kin discrimination (nepotism) could select for indiscriminate acceptance of eggs (Keller 1997; Holzer et al. 2006).

Material and methods

Study species and egg source

Our study population of the ant *F. selysi* is located along the river Rhône in central Valais, Switzerland (7°36′30″E, 46° 18′30″N, altitude 565 m). The social structure (monogyne or polygyne) of each colony used in this experiment had been previously determined by genotyping eight to 100 workers at nine microsatellite markers (Chapuisat et al. 2004; Schwander et al. 2005; Rosset and Chapuisat 2006; Reber et al. 2008).

Queens in monogyne colonies from our study population are generally singly mated (Chapuisat et al. 2004). Hence, workers from monogyne colonies are on average more related to the sons of other workers than to the sons of the queens. In polygyne colonies, the relatedness towards males depends on the number of queens and relatedness among queens. In general, workers are also more related to the sons of other workers than to the sons of the queens if colonies contain relatively few queens of low relatedness, or when colonies recruit new queens from their own daughters (Pamilo 1991; Crozier and Pamilo 1996). Our microsatellite data from nine polygyne colonies indicate that workers have a similar degree of relatedness to the queens' sons and to the expected sons of workers (0.067± 0.04 and 0.065±0.01, respectively, mean ± SE); (Chapuisat et al. 2004; Rosset and Chapuisat 2006). Overall, if worker policing is only caused by relatedness asymmetry, it should be rare or absent in our study population, particularly in monogyne colonies.

Queen-laid eggs were sampled from 23 single-queen and 17 polygyne field colonies during the first week of May 2008. These eggs were the first ones produced in the season, and they develop into queens or males, whereas workers are produced later in the season (Rosset and Chapuisat 2006). We assume that eggs collected in field colonies are queen-produced, because we had previously shown that worker reproduction was absent or very rare in queenright field colonies of our study population. Indeed, we did not detect any worker-produced eggs when genotyping 341 male eggs originating from 27 monogyne queenright field colonies (Rosset and Chapuisat 2006).

Worker-laid eggs were obtained from groups of workers that were experimentally separated from their queens. We sampled 200 workers from each of 25 monogyne and 20 polygyne field colonies during the second week of April 2008 and transferred them to the laboratory. Thirty groups of queenless workers (originating from 16 and 14 monogyne and polygyne colonies, respectively) produced more than 20 eggs, and could thus be included in the egg-acceptance bioassay. We standardized the age of the eggs used in the egg-acceptance bioassays by performing all assays approx-

imately 10 days after queens and workers had started to lay (that is, between April 30th and May 7th 2008).

Egg-acceptance bioassays

We estimated the collection rate and survival rate of eggs introduced into recipient groups of workers. The recipient workers were sampled between April 30th and May 7th 2008 from 29 and 25 monogyne and polygyne field colonies, respectively. They were distributed in 102 recipient groups, of which 57 and 45 originated from monogyne and polygyne field colonies, respectively (see Figs. 1 and 2 for sample sizes—there were up to three recipient groups per colony, each receiving a set of eggs of different origin). Each recipient group was composed of 100 workers placed in a small plastic box (15 × 15 × 5 cm) lined with fluon. Each box contained one test tube with wet cotton wool that workers used as nest. Workers had ad libitum access to standard ant food (for food composition see Meunier and Chapuisat 2009). To limit the influence of orphaning on workers responses, egg-acceptance bioassays were performed 16 h after the sampling of workers in the field.

Each recipient group of workers received a set of 29±7 (mean ± SD) eggs laid either by (1) nestmate workers, (2) nestmate queens, or (3) queens originating from a foreign colony of the same social structure as the recipient workers. Prior to introduction, we placed eggs in small plastic trays (3×3 cm) and observed them under a stereomicroscope to check that they were not damaged. We then transferred the trays with eggs into recipient groups.

We estimated egg collection rate in a subsample of 66 recipient groups by counting the number of eggs remaining on the trays 15 min after their introduction in recipient groups (see Fig. 1 for sample sizes). We estimated egg survival rate by counting the total number of undamaged eggs present in each recipient group of workers 24 h after introduction (see Fig. 2 for sample sizes).

Experimental controls

We controlled for two factors that could influence the elimination of worker-laid eggs, namely their laboratory origin and sex. To examine if eggs laid in laboratory colonies were treated differently from eggs laid in field colonies, we compared the response of workers towards foreign queen-laid eggs produced in the laboratory and in the field. To this end, we collected workers from nine polygyne field colonies in the first week of April 2008. Each of these nine additional groups of recipient workers received 30 eggs laid by foreign queens in the laboratory. These queens originated from nine polygyne field colonies and had been kept in the laboratory since March 2007. We then estimated egg collection rate and egg survival rate as described above.

We also examined if differences in the sex of eggs could influence the discrimination of worker-laid and queen-laid eggs. Worker-laid eggs are exclusively haploid (male destined), whereas queen-laid eggs can be a mix of haploid and diploid (male and female destined) eggs. We controlled for this potential effect of sex by restricting one comparison to haploid eggs only. In our study population, the proportion of male pupae produced by field colonies depends on the proportion of haploid eggs laid by the queens (Rosset and Chapuisat 2006). In particular, some of the field colonies specialize in the production of males and

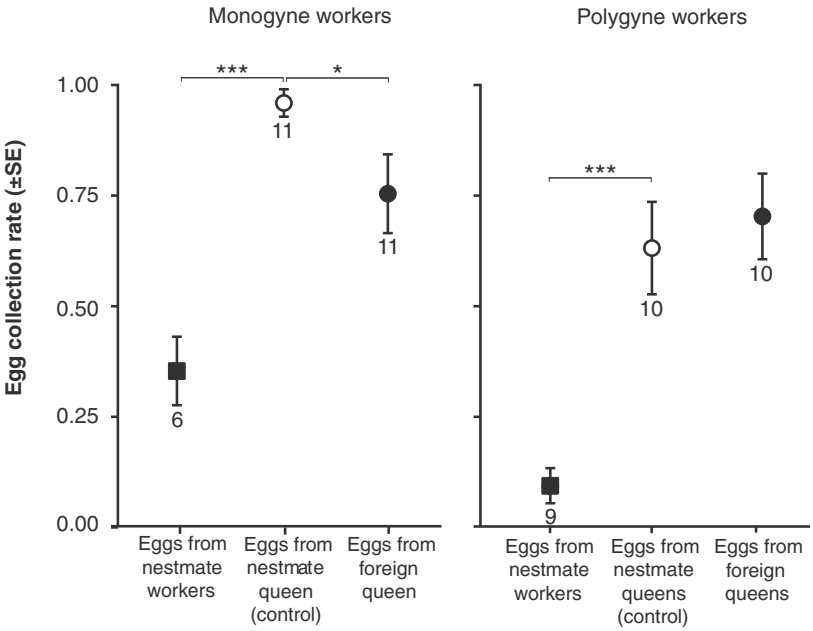

Fig. 1 Collection rates of eggs originating from nestmate workers (*black squares*), nestmate queens (control, *white circles*) or foreign queens (*black circles*) 15 min after being introduced in group of monogyne or polygyne workers. The number of recipient groups of workers is indicated below the SE bars. *$P<0.05$, ***$P<0.001$

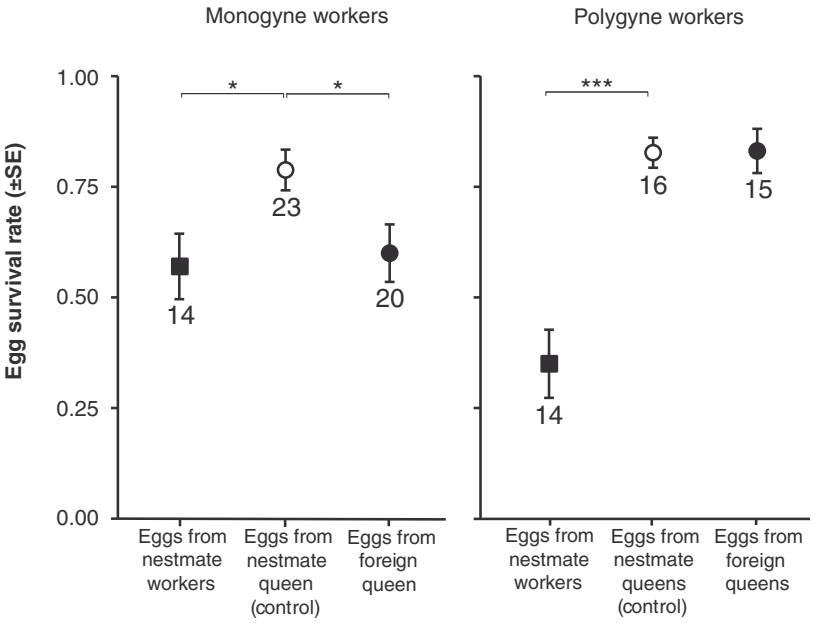

Fig. 2 Survival rates of eggs originating from nestmate workers (*black squares*), nestmate queens (control, *white circles*), or foreign queens (*black circles*) 24 h after being introduced in group of monogyne or polygyne workers. The number of recipient groups of workers is indicated below the SE bars. *$P<0.05$, ***$P<0.001$

contain only haploid, male-destined queen-laid eggs at this time of the year (Rosset and Chapuisat 2006). We determined the sex of 30±11 (mean ± SD) pupae in 22 of the field colonies that were used as source of queen-laid eggs, and identified nine male-specialized colonies, six monogyne, and three polygyne ones, respectively. To examine if workers discriminated worker-laid eggs from queen-laid eggs independently of the sex of eggs, we compared the survival of haploid worker-laid eggs to the ones of haploid queen-laid eggs originating from this subsample of male-specialized field colonies.

Statistical analysis

We tested if workers discriminated between worker-laid and queen-laid eggs in a two-way analysis of variance (ANOVA). The maternal origin of eggs (workers or queens) and the social structure of workers (monogyne or polygyne) were used as fixed factors. The response variables were the proportion of eggs collected from the trays 15 min after introduction (egg collection rate) and the proportion of eggs still alive after 24 h (egg survival rate). Because eggs or workers originating from the same field colony were sometimes used twice in the analyses (albeit once per type of eggs assayed), we included the colony of origin of eggs and workers as random factors in all the ANOVAs. To test whether workers discriminated between foreign and nestmate queen-laid eggs, we used a similar two-way ANOVA in which the origin of eggs (i.e., nestmate or foreign) and the social structure of workers were used as fixed factors.

In addition, we performed pairwise comparisons between control eggs (nestmate queen-laid eggs) and treatment eggs (worker-laid eggs or foreign queen-laid eggs) within groups of recipient workers originating from each social structure (monogyne or polygyne). We used one-way ANOVAs in which the significance level was Bonferroni-adjusted to $\alpha=0.025$.

We compared the survival rate of haploid eggs laid by workers and by nestmate queens using non-parametric Wilcoxon Rank Sum tests. Variables were normalized by arcsine transformations (Sokal and Rohlf 1995). All statistical analyses were conducted using the computer program JMP 7.0 (2007, SAS Institute, http://www.jmp.com).

Results

Workers from both monogyne and polygyne colonies discriminated against worker-laid eggs (Figs. 1 and 2, Table 1a). Indeed, in both types of colonies worker-laid eggs were collected by workers at a slower rate and had a lower survival rate than queen-laid eggs (pairwise comparisons; monogyne workers: collection rate: $F_{1,15}=55.27$, $P<0.0001$, and survival rate: $F_{1,29.81}=7.21$, $P=0.012$; polygyne workers: collection rate: $F_{1,17}=16.78$, $P=0.0001$, and survival rate: $F_{1,21.38}=22.02$, $P<0.0001$). Monogyne workers collected both worker-laid and queen-laid eggs at a faster rate than polygyne workers, whereas the egg survival rate did not differ significantly between the two types of workers (Table 1a). There was no significant interaction between the maternal origin of eggs (worker or queen) and the social origin of the recipient workers (monogyne or polygyne colonies; Table 1a). This suggests that workers originating from monogyne and polygyne colonies discrim-

Table 1 Effects of the social origin of workers on the discrimination between eggs laid by nestmate workers and nestmate queens, as well as between eggs laid by nestmate queens and foreign queens originating from a colony of the same social structure as the workers

	Egg collection rate		Egg survival rate	
Worker-laid vs. queen-laid eggs				
Origin of eggs	$F(1,32)=53.73$	**$P<0.0001$**	$F(1,51.81)=28.02$	**$P<0.0001$**
Social origin of workers	$F(1,32)=16.42$	**$P=0.0003$**	$F(1,42.14)=2.53$	$P=0.119$
Interaction	$F(1,32)=0.68$	$P=0.416$	$F(1,51.81)=2.73$	$P=0.105$
Nestmate vs. foreign queen-laid eggs				
Origin of eggs	$F(1,19)=1.64$	$P=0.215$	$F(1,40.35)=2.38$	$P=0.131$
Social origin of workers	$F(1,19)=4.12$	$P=0.057$	$F(1,40.92)=6.22$	**$P=0.017$**
Interaction	$F(1,19)=7.29$	**$P=0.014$**	$F(1,40.35)=4.86$	**$P=0.033$**

Significant results are in bold

inated worker-laid from queen-laid eggs in similar ways (Figs. 1 and 2).

The differential treatment of worker-laid and queen-laid eggs could not be explained by a discrimination against eggs laid under laboratory conditions or a discrimination against haploid eggs. The rate at which eggs from foreign queens were collected and survived did not differ significantly between eggs produced in laboratory colonies (mean ± SE= 0.54±0.10 and 0.82±0.04, respectively; n=9 colonies) and eggs sampled in field colonies (Figs. 1 and 2; one-way ANOVAs, collection rate: $F_{1,9.19}$=0.926, P=0.361; Survival rate: $F_{1,11.26}$=0.29, P=0.602). The workers also distinguished between haploid eggs laid by nestmate queens or nestmate workers. Indeed, the survival of haploid queen-laid eggs originating from male-specialized field colonies (monogyne workers: 0.86±0.06, n=6; polygyne workers: 0.79±0.04, n=3) was significantly higher than the one of haploid worker-laid eggs (Fig. 2; monogyne workers: W=71.5, P=0.015; polygyne workers: W=37, P=0.043)

Only workers from monogyne colonies discriminated against foreign queen-laid eggs, which resulted in a significant interaction between the origin of eggs (nestmate or foreign queen) and the social origin of workers (monogyne or polygyne colonies; Table 1b). In groups of monogyne workers, eggs laid by nestmate queens were collected at a faster rate and survived better than eggs laid by foreign queens (Figs. 1 and 2; pairwise comparisons, collection rate: $F_{1,10}$=5.44, P=0.042; survival rate: $F_{1,20.02}$=6.10, P= 0.021). In contrast, in groups of polygyne workers, eggs laid by nestmate and foreign queens were collected and survived at similar rates (collection rate: $F_{1,9}$=2.34, P= 0.161; Survival rate: $F_{1,15.71}$=0.46, P=0.508).

Discussion

Our egg-acceptance bioassays show that *F. selysi* workers from monogyne and polygyne colonies discriminated eggs laid by nestmate workers from eggs laid by nestmate queens, even when only haploid eggs were compared. The lower collection rate and survival of worker-laid eggs suggest that workers police worker-laid eggs in both types of colonies. These signs of worker policing in monogyne colonies do not support the hypothesis that policing is only due to relatedness asymmetry, as workers in monogyne colonies are more related to workers' sons than to queens' sons, and should thus allow worker reproduction if it has no other cost.

The discrimination against worker-laid eggs is consistent with the hypothesis that worker policing evolved because worker reproduction decreases colony efficiency and productivity (Ratnieks 1988; Hammond and Keller 2004; Wenseleers and Ratnieks 2006). Theoretically, fairly small colony-level costs due to worker reproduction may suffice to compensate for the relatedness asymmetry and select for worker policing, even in colonies with one singly mated queen (Ratnieks 1988). However, these costs are difficult to estimate empirically, as colony fitness depends upon the cumulative effect of many variables, such as the amount of brood produced, colony size, or foraging success (Cole 1986; Gobin et al. 2003; Dijkstra and Boomsma 2007). One of the best demonstrations that worker policing limits the colony-level costs of worker reproduction came from the ant *Platythyrea punctata* (Hartmann et al. 2003). Because these ants are clonal, there is no relatedness asymmetry. The aggression of workers against additional reproducing workers helps to maintain a low number of reproducing individuals and contributes to increase colony efficiency by limiting the number of brood (Hartmann et al. 2003).

The discrimination between worker-laid and queen laid eggs is unlikely to be due to a selective elimination of haploid eggs, a lower intrinsic viability of worker-laid eggs, or a selective removal of eggs laid under laboratory conditions. Workers might be able to distinguish between haploid and diploid eggs, and may selectively eliminate haploid brood (Rosset and Chapuisat 2006). However, in our experiments, workers distinguished haploid worker-laid eggs from haploid queen-laid eggs, which indicate that the discrimination against worker-laid eggs can occur independently from a potential ability to distinguish haploid from diploid eggs. With respect to egg viability, the eggs remained in recipient colonies for only 24 h. Moreover,

worker-laid eggs and queen-laid eggs kept in similar queenless groups readily developed into adult males (Joël Meunier, unpublished results; Meunier and Chapuisat 2009). A study in *A. mellifera* also suggests that workers do not use egg viability to recognize worker-laid eggs, as workers did not discriminate between viable and CO_2-killed queen-laid eggs, whereas they did selectively eliminate worker-laid eggs (Beekman and Oldroyd 2005). Lastly, a rejection of eggs because of laboratory conditions is unlikely, as we found that the collection and survival rates of eggs laid by queens in laboratory colonies did not differ significantly from the ones of eggs laid by queens in field colonies.

Somewhat surprisingly, the collection rate of nestmate eggs was higher in groups of monogyne workers than in groups of polygyne workers. Monogyne workers might be quicker than polygyne ones because they have a larger body size (Schwander et al. 2005). Alternatively, monogyne eggs might be easier to handle because of their smaller volume (Meunier and Chapuisat 2009).

In contrast to worker-laid eggs, eggs laid by foreign queens were only discriminated against by workers from monogyne colonies, but not by workers from polygyne colonies. The ability of monogyne workers to distinguish eggs laid by foreign monogyne queens from eggs laid by nestmate queens shows that nestmate discrimination of queen-laid eggs occurs in *F. selysi*. A discrimination against foreign eggs has been recently documented in another ant species of the same genus (Helanterä and Sundström 2007a). The fact that workers have some ability to discriminate against eggs laid by foreign queens might contribute to maintain the genetic integrity of monogyne colonies. It also challenges the conventional view that there is no nestmate recognition of queen-laid eggs in Hymenoptera and that queen-laid eggs are universally accepted because they are marked by a conserved queen-produced pheromone (e.g., Ratnieks and Visscher 1989; Ratnieks and Boomsma 1995; Martin et al. 2002; Pirk et al. 2003; Endler et al. 2004).

To our knowledge, our study is the first demonstration that variation in the number of queens per colony influences the ability of workers to discriminate against foreign eggs in social Hymenoptera. The discrimination against foreign eggs in one type of social structure and not the other indicates that egg recognition is affected by colony kin structure. It is likely that variation in the number of queens per colony affects the diversity and the use of cues involved in the discrimination of eggs.

The absence of discrimination against foreign queen-laid eggs in polygyne colonies might be due to larger costs of nestmate discrimination in polygyne colonies, as compared to monogyne ones. The presence of multiple queens broadens the template of cues used by workers for nestmate recognition and consequently increases the risk of costly discrimination errors (Bourke and Franks 1995; Vander Meer and Morel 1998; Helanterä and Ratnieks 2009). In addition, workers' ability to discriminate between eggs from different matrilines might lead to nepotistic behaviors within polygyne colonies, which would probably decrease the colony productivity and thus inclusive fitness of all colony members (Keller 1997; Holzer et al. 2006; Ratnieks et al. 2006).

Our data do not allow determining whether the absence of nestmate discrimination in polygyne colonies results from a lack of discrimination ability in polygyne workers or a lack of colony-specific cues on eggs laid by polygyne queens. In order to distinguish between these hypotheses, it would be of interest to investigate the chemical cues on eggs and to perform further experiments on the ability of monogyne and polygyne workers to discriminate against foreign queen-laid eggs originating from the alternative social structure.

The effect of social structure variation on nestmate discrimination seems to be context-dependent in our study population. Indeed, workers from monogyne and polygyne colonies discriminated against foreign workers and foreign queens, whatever their social structure of origin (Rosset et al. 2006; Joël Meunier, Anabelle Reber, and Michel Chapuisat, unpublished results). Moreover, workers from both types of colonies discriminated against worker-laid eggs. This suggests that the recognition of nestmate queen-laid eggs, nestmate adults and worker-laid eggs are partially independent in *F. selysi*. Interestingly, workers also used distinct processes to discriminate foreign from nestmate queen-laid eggs and worker-laid from queen-laid eggs in the ant *F. fusca* (Helanterä and Ratnieks 2009).

In conclusion, our results show that *F. selysi* workers have a well-developed and context-dependent ability to discriminate between eggs from various origins. The selective elimination of worker-laid eggs occurs in monogyne and polygyne colonies, which is consistent with the hypothesis that workers police each others' eggs to avoid the colony-level productivity costs of worker reproduction. In contrast, only monogyne workers discriminated eggs laid by foreign monogyne queens from eggs laid by nestmate queens, whereas polygyne workers did not make this distinction. Workers in polygyne colonies are probably exposed to a larger diversity of cues, and their discrimination ability might be restricted in order to prevent the emergence of nepotistic conflicts among matrilines. This conditional influence of social structure variation on egg discrimination by workers is a novel example of the intricate processes modulating recognition in a social context.

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Split sex ratios in the social Hymenoptera: a meta-analysis

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The study of sex allocation in social Hymenoptera (ants, bees, and wasps) provides an excellent opportunity for testing kin-selection theory and studying conflict resolution. A queen–worker conflict over sex allocation is expected because workers are more related to sisters than to brothers, whereas queens are equally related to daughters and sons. If workers fully control sex allocation, split sex ratio theory predicts that colonies with relatively high or low relatedness asymmetry (the relatedness of workers to females divided by the relatedness of workers to males) should specialize in females or males, respectively. We performed a meta-analysis to assess the magnitude of adaptive sex allocation biasing by workers and degree of support for split sex ratio theory in the social Hymenoptera. Overall, variation in relatedness asymmetry (due to mate number or queen replacement) and variation in queen number (which also affects relatedness asymmetry in some conditions) explained 20.9% and 5% of the variance in sex allocation among colonies, respectively. These results show that workers often bias colony sex allocation in their favor as predicted by split sex ratio theory, even if their control is incomplete and a large part of the variation among colonies has other causes. The explanatory power of split sex ratio theory was close to that of local mate competition and local resource competition in the few species of social Hymenoptera where these factors apply. Hence, three of the most successful theories explaining quantitative variation in sex allocation are based on kin selection. *Key words:* meta-analysis, queen–worker conflict, relatedness asymmetry, sex allocation, social insects, split sex ratio. *[Behav Ecol 19:382–390 (2008)]*

Kin selection extends natural selection to include the indirect transmission of copies of genes through relatives (Hamilton 1964). This theory is fundamental to understanding a wide variety of evolutionary phenomena such as the evolution of altruism and spite, the emergence of eusociality, and the presence of kin conflicts (Hamilton 1964, 1970, 1972; Bourke and Franks 1995; Gardner and West 2004; Ratnieks et al. 2006; West et al. 2007). Some of the clearest opportunities for testing kin-selection theory are provided by conflicts over sex allocation in the social Hymenoptera (Trivers and Hare 1976; Bourke and Franks 1995; Crozier and Pamilo 1996; Chapuisat and Keller 1999). Social Hymenoptera are haplodiploid with diploid females produced from fertilized eggs and haploid males from unfertilized ones. This sex-determination system results in relatedness asymmetries between workers (females who raise the brood) and sexual individuals (queens and males). When colonies are headed by 1 single-mated queen, workers are 3 times more related to sisters than to brothers, whereas queens are equally related to daughters and sons (Trivers and Hare 1976). Hence, kin selection predicts a potential conflict between queens and workers, with queens favoring a balanced sex allocation and full-sibling workers a 3 times larger investment in females than in males.

The quantitative predictions vary with changes in social structure, which affect relatedness asymmetry. Specifically, relatedness asymmetry is expected to decrease when (i) the queens mates with more than one male, (ii) the queen is replaced by one of her daughters, (iii) multiple related queens reproduce in the same colony, and (iv) workers produce males (Hamilton 1972; Trivers and Hare 1976; Boomsma and Grafen 1990, 1991; Boomsma 1991, 1993; Foster and Ratnieks 2001). Under worker control, these changes in relatedness asymmetry should result in less female-biased sex allocation relative to the case with 1 single-mated queen, and on average, the degree of queen–worker conflict should decrease.

Variation in relatedness asymmetry can occur among species, among populations, and among colonies within populations. The comparison of sex allocation and relatedness asymmetry across ant species and populations provides evidence for partial worker control, with female-biased sex allocation in species that have a single queen per colony (monogyne colonies) and slightly male-biased sex allocation in species with multiple queens per colony (polygyne colonies; Trivers and Hare 1976; Pamilo and Rosengren 1983; Nonacs 1986a; Pamilo 1990; Bourke 2005). However, this pattern is open to multiple explanations due to correlated factors. In particular, queens from polygyne colonies often stay in their natal nest while males disperse, and this local resource competition (LRC) among queens also promotes male-biased sex allocation independently of the decrease in relatedness asymmetry (Crozier and Pamilo 1996; Chapuisat and Keller 1999).

The most powerful method for testing the queen–worker conflict over sex allocation is to examine if sex ratio is split according to relatedness asymmetry variation among colonies within populations. The theory predicts that under worker control, colonies with relatively high or low relatedness asymmetry should specialize in producing females or males, respectively (Boomsma and Grafen 1990, 1991; Boomsma 1991). In as many as 19 out of 25 species or populations studied so far, colony sex allocation is indeed split according to measured or putative variation in relatedness asymmetry (Queller and Strassmann 1998; Chapuisat and Keller 1999; Mehdiabadi et al. 2003; Bourke 2005). This general pattern is consistent

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with widespread worker control and provides strong qualitative support to kin-selection theory (Queller and Strassmann 1998; Chapuisat and Keller 1999; Bourke 2005). However, the magnitude of adaptive sex allocation biasing by workers has not been quantified so far.

In this study, we performed a meta-analysis on empirical tests of split sex ratio theory. Our first aim was to use all published studies to quantify the impact of worker control over colony sex allocation in the social Hymenoptera. Our second aim was to examine if the degree of sex allocation adjustment depends on the source and/or magnitude of relatedness asymmetry variation. Relatedness asymmetry can vary among colonies because of queen replacement, variation in queen mating frequency, and variation in queen number under certain conditions. All types of studies have been used to qualitatively test split sex ratio theory, but they are likely to differ with respect to information constraints and strength of selection on worker behavior, which depends on the magnitude of variation in relatedness asymmetry (Boomsma et al. 2003; Bourke 2005). For example, the replacement of a queen by one of her daughters is probably easy to detect by workers, and it results in the highest decrease in relatedness asymmetry (3:1 to 1:1). In contrast, workers might have more difficulty to assess the number of males that have mated with the queen because queens mate before the birth of the workers and store the sperm for the rest of their life. Workers therefore have to infer mate number from the level of colony genetic diversity, which might be a difficult task particularly if the cost of nepotistic behavior selects against genetically based odor cues (Boomsma et al. 2003). Higher number of queens also decreases relatedness asymmetry when queens are related (Boomsma 1993; Bourke and Franks 1995). However, changes in relatedness asymmetry might be small, continuous, and somewhat erratic because of the dynamics of queen replacement, and therefore they are likely to be difficult to assess for workers in polygyne colonies. Our third aim was to compare the explanatory power of split sex ratio theory to the other most successful areas of sex allocation—local mate competition (LMC, which predicts a bias toward females when related males compete over access to females; Hamilton 1967) and LRC (which predicts a bias toward males when related females compete over resources; Clark 1978).

METHODS

Collection of data

We performed a large-scale search for studies that contained relevant data and read abstracts to select studies on social Hymenoptera. We combined several methods: (1) searching for references in reviews of the subject (Herbers 1979; Nonacs 1986a, 1986b; Bourke and Franks 1995; Crozier and Pamilo 1996; Queller and Strassmann 1998; Chapuisat and Keller 1999; Mehdiabadi et al. 2003; Bourke 2005; Ratnieks et al. 2006); (2) searching the Institute for Scientific Information Web of science on 7 May 2007 for all articles containing at least one of the following expressions: "sex ratio variation," "relatedness asymmetry," "sex investment ratio," "queen mating," "monoandrous," "monandrous," "polyandrous," or "split sex ratio"; and (3) searching citations in all papers found by the above method. We obtained more than 700 studies out of which 27 were relevant for our aims.

We did not include studies for which appropriate effect sizes could not be calculated, such as studies without data on both colony sex allocation and variation of colony relatedness asymmetry or breeding system (Brian 1979; Pamilo and Rosengren 1983; Ward 1983; Strassmann 1984; Elmes 1987; Herbers 1990; Stark 1992; Fuchs and Schade 1994; Vargo 1996; Helms 1999). Because the meta-analysis requests an estimate of the correlation between sex allocation and relatedness asymmetry, we had to exclude studies in which there was no variation in relatedness asymmetry among colonies within populations (Passera et al. 2001; Duchateau et al. 2004). We also excluded studies based on experimentally selected colonies (Kikuchi et al. 2002) or on worker relatedness without information on queen number, queen relatedness, or queen mating frequency in a slave-making ant species (Pamilo and Seppä 1994). In few cases, we contacted the authors to obtain additional information on published data sets (Yanega 1989; Queller et al. 1993; Pearcy and Aron 2006).

We separately collected studies that investigated the impact of competitive interactions among relatives on sex allocation in social Hymenoptera. We used the data set of West et al. (2005), complemented by searching the Institute for Scientific Information Web of science on 7 May 2007 for all articles containing at least one of the expressions "local resource competition" or "local mate competition" in social Hymenoptera. As a result, we added 3 new studies published since 2005 to the 9 studies on social Hymenoptera reviewed in West et al. (2005).

Data analysis

We analyzed our data using meta-analysis methods, where the calculated effect size of each study is used as a response variable in a global analysis (Rosenthal 1991; Rosenberg et al. 2000). Each effect size (r) is a correlation coefficient providing an estimate of how colonies adjust their sex allocation in response to relatedness asymmetry variation, queen number variation, or competitive interactions (LRC plus LMC). We defined a positive effect size when colonies with higher relatedness asymmetry (or smaller queen number, lower LRC, and higher LMC) had a more female-biased sex allocation and negative when colonies with lower relatedness asymmetry (or larger queen number, higher LRC, and lower LMC) had a more female-biased sex allocation. Hence, a positive, large effect size indicates that sex allocation followed the predicted pattern.

We calculated effect sizes using standard methodology (Rosenthal 1991; Rosenberg et al. 2000). The values sometimes come from the Spearman rank correlation coefficient (r_s) provided in the publication. In other cases, the effect size could be calculated from the statistics (e.g., t, χ^2, F, Z, or P values) and sample size using standard formulas (Rosenthal 1991; Rosenberg et al. 2000). If the test statistics were derived from the analysis of variance (ANOVA) with more than 2 treatments, we applied an ordered heterogeneity (OH) test (see Rice and Gaines 1994). Finally, when values were not available, we used raw data given in figures or tables. The proportion of variance in colony sex allocation that is explained by the factor is given by r^2.

All analyses were performed using the software package Metawin 2.0 (Rosenberg et al. 2000) with random-effect model (Møller and Jennions 2002; West et al. 2005) and the statistical software R.2.5.0 (Ihaka and Gentleman 1996). Statistical analyses were conducted on Z-transformed r values (Zr) to correct for asymptotic behavior of large values of r (Sheldon and West 2004), and the bias-corrected 95% confidence interval (CI) were obtained by bootstrapping (Rosenberg et al. 2000). We tested for statistical differences between the mean effect sizes with randomized ANOVA in which effect sizes were randomly permuted 10 000 times between factors (Manly 1997). Results were back transformed to r values for presentation.

We conducted each analysis with 1 mean effect size per species in each factor category (relatedness asymmetry variation, queen number variation, and competitive interactions

Table 1

Mean effect sizes of studies investigating sex allocation adjustment in response to relatedness asymmetry variation, queen number variation, and competitive interaction among relatives

Class of study Factor	Mean effect size (r)	95% CI	Number of species
Relatedness asymmetry variation	**0.457****	**0.211–0.674**	**7**
Queen replacement	0.552**	0.300–0.786	3
Mate number	0.368*	0.003–0.648	4
Queen number variation	**0.223****	**0.107–0.323**	**15**
Monogyne versus polygyne colonies	0.090	−0.216–0.320	9
Count of queens in polygyne colonies	0.240**	0.071–0.426	4
From relatedness variation	0.354**	0.292–0.484	6
Competitive interactions among relatives	**0.501****	**0.375–0.619**	**10**
LRC	0.496**	0.285–0.660	7
LMC	0.473**	0.222–0.601	4

Asterisks indicate effect sizes that are significantly greater than 0 ($^{*}P < 0.05$, $^{**}P < 0.01$).

among relatives; Table 1 and Appendix). When the same species was studied in several populations or over several years, we calculated an average Zr, weighted by sample size. We summed up sample sizes when different colonies were sampled and calculated an average when the same colonies were sampled repeatedly.

We used several methods to detect a potential publication bias—a tendency to be more likely to publish studies with significant results. First, we plotted the effect sizes against sample sizes. In the absence of publication bias, the plot should have a funnel shape with the values of effect sizes equilibrating to the average when sample size increases (Møller and Jennions 2001). In contrast, a significant negative correlation between effect size and sample size suggests that studies with significant results have been preferentially published, which causes a deficit of studies with nonsignificant results and small sample sizes. Second, we applied the "trim and fill" method to evaluate the bias in the funnel plot and the significance of the result (Johnson et al. 2000). This method estimates the number (L_0) and effect size of studies that are missing from a meta-analysis due to publication bias and then adds them to the data set, recalculates the mean effect size, and derives its statistical significance (Møller and Jennions 2002). Finally, we calculated the "fail-safe number" (X), which is the number of unpublished studies with an effect size of 0 that would be needed to change the result from significant to not significant (Rosenthal 1991). Interpretation of the meaning of X depends in part on the subjective assessment of whether so many unpublished studies are likely to exist. A quantitative criterion is that a result is robust if $X > 5n + 10$, where n is the number of studies on which the meta-analysis was based, although this criterion is hard to meet with small sample sizes (Rosenberg et al. 2000).

We investigated whether the degree of worker control was linked to the magnitude of relatedness asymmetry variation between colonies in the population. This magnitude of relatedness asymmetry variation was estimated as the proportion $\frac{RA_{high}}{RA_{high} + RA_{low}}$ in which RA_{high} and RA_{low} were mean relatedness asymmetries in the highest and lowest relatedness asymmetry classes, respectively (see Appendix for details). In some cases, these relatedness asymmetries were directly measured with microsatellite or allozyme markers. In other cases, they were inferred from social structure variation (mate number, mother or sister queen, queen number). The relatedness asymmetry within polygyne colonies was estimated as $1 + \frac{2}{1 + r_q(n-1)}$, where n is the number of queens and r_q the relatedness among queens that we assumed to be equal to the relatedness among workers because queens are usually recruited back into their natal colony in species with polygyne colonies (Boomsma 1993; Crozier and Pamilo 1996). When not available, the number of queens was estimated as $\frac{3-r_q}{\left(r_w - \frac{r_q}{4}\right)\times 4}$, where r_w is the relatedness among workers (Hughes et al. 1993; Boomsma 1993).

RESULTS

Relatedness asymmetry variation

We found 7 studies with quantitative data on sex allocation adjustment in response to relatedness asymmetry variation among colonies due to queen replacement or mate number variation (Appendix). Data on queen replacement by daughter were available for 3 species of sweat bees, and data on mate number variation were available for 3 ant and 1 bumblebee species.

Overall, sex allocation was significantly correlated with relatedness asymmetry, in the direction predicted by worker control, with a mean effect size of $r = 0.457$ (Table 1). Hence, worker control according to relatedness asymmetry explains 20.9% of the variance in sex allocation. The extent of sex allocation adjustment did not depend on the cause of relatedness asymmetry variation. Specifically, there was no significant difference between the mean effect size of studies on queen replacement by daughter ($r = 0.552$) and variation in mate number ($r = 0.368$; randomized ANOVA, $n = 7$, $P = 0.54$). However, the number of species studied was small and the trend was in the direction predicted by the information constraints, which are higher for mate number variation than queen replacement.

The effect sizes were highly variable but seemed to be uniformly distributed and showed no sign of a publication bias (Figure 1). The trim and fill analysis did not detect missing studies ($n = 7$, number of missing studies $L_0 = 0$), and there was no significant correlation between effect size and sample size (Spearman rank correlation test, $n = 7$, $r_s = -0.036$, $P = 0.94$). The fail-safe number was small ($X = 15$, quantitative criterion $= 45$), but the criterion is extremely hard to meet with small sample sizes (Rosenberg et al. 2000).

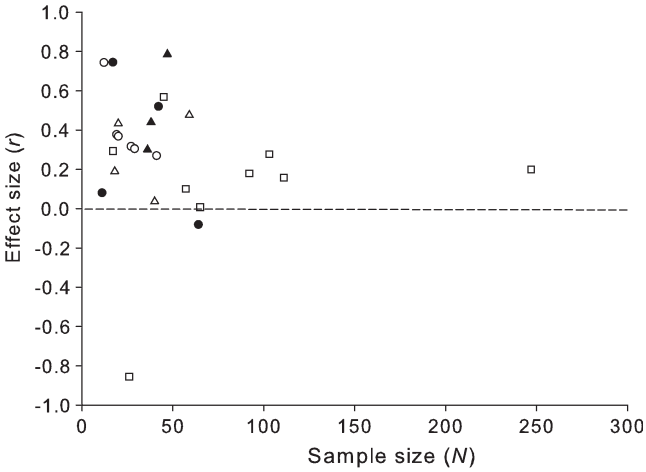

Figure 1
Relationship between effect size and sample size for studies on queen replacement by daughter (filled triangles), variation in mate number (filled circles), comparison between monogyne and polygyne colonies (open squares), queen number variation inferred from the count of queens in polygyne colonies (open triangles), and queen number variation inferred from relatedness (open circles). Each point corresponds to 1 species.

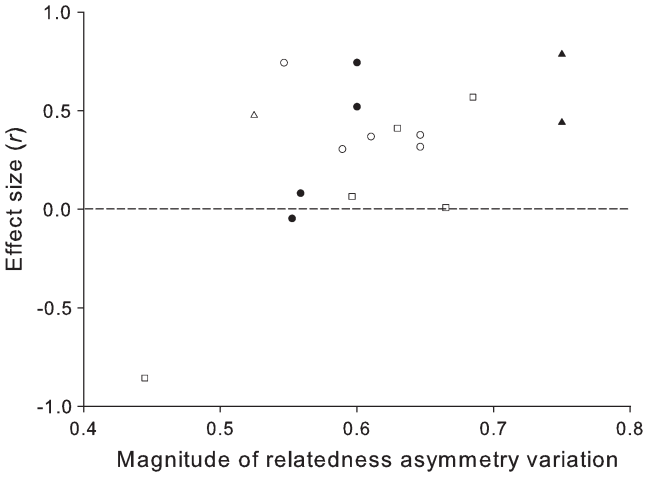

Figure 2
Relationship between effect size and magnitude of relatedness asymmetry variation for studies on queen replacement by daughter (filled triangles), variation in mate number (filled circles), comparison between monogyne and polygyne colonies (open squares), queen number variation inferred from count of queen in polygyne colonies (open triangle), and queen number variation inferred from relatedness (open circles).

Queen number variation

We found 16 studies examining sex allocation adjustment and queen number variation among colonies (Appendix). There were data on sex allocation in monogyne and polygyne colonies for 8 ant and 1 wasp species, data on the correlation between sex allocation and counts of queens in polygyne colonies for 4 ant species, and data on sex allocation with respect to queen number inferred from relatedness variation for 1 ant and 5 wasp species.

Overall, sex allocation was significantly correlated with queen number variation, in the predicted direction that colonies with higher queen numbers produced more males (Table 1, $r = 0.22$). Hence, changes in queen number explained on average 5% of the variance in sex allocation. This value is conservative because 6 out of the 15 effect sizes had to be calculated from P value thresholds or without applying OH test, resulting in slightly underestimated effect sizes (see Appendix).

The impact of queen number variation on sex allocation did not depend on the group of study. The mean effect size of the comparison between monogyne and polygyne colonies, queen number variation in polygyne colonies, and queen number variation inferred from relatedness were not significantly different (randomized ANOVA, $n = 19$, $P = 0.263$). It is possible that the comparison between monogyne and polygyne colonies had a lower and nonsignificant effect size because polygyne colonies can occasionally be headed by unrelated queens and thus have high relatedness asymmetries (e.g. Fournier et al. 2003). However, interpreting the differences is delicate as there was overall no significant difference between the mean effect sizes of relatedness asymmetry variation and queen number variation (randomized ANOVA, $n = 22$, $P = 0.20$).

The mean effect size became nonsignificantly different from 0 when carrying out a trim and fill analysis ($n = 15$, number of missing studies $L_0 = 5$, adjusted mean $r = 0.132$, 95% CI: 0.08–0.253, $P > 0.05$). This small, adjusted mean effect size might be partly due to the 6 studies for which the effect size was slightly underestimated (see Appendix). Otherwise, there was no significant correlation between sample size and effect size (Spearman rank correlation test, $n = 15$, $r_s = -0.357$, $P = 0.19$) and the fail-safe number was large ($X = 78$, quantitative criterion = 115).

Effect size and magnitude of relatedness asymmetry variation

The magnitude of variation in relatedness asymmetry among the most differing classes of colonies could be measured for 17 studies (Appendix). Overall, it was not significantly correlated with effect size (Figure 2, Spearman rank correlation test, $n = 17$, $r_s = 0.331$, $P = 0.20$). This nonsignificant pattern held when analyses were restricted to studies on queen replacement and mate number variation (Spearman rank correlation test, $n = 6$, $r_s = 0.736$, $P = 0.10$) or queen number variation (Spearman rank correlation test, $n = 11$, $r_s = 0.10$, $P = 0.77$). It should, however, be noted that these analyses are based on small data sets and that the correlations tend to be positive, particularly in the case of queen replacement and mate number variation.

Competitive interactions among relatives

We found 12 studies on 10 species examining how competitive interactions among relatives influence sex allocation in the social Hymenoptera. These involved 7 studies on LRC and 6 studies on LMC, in 10 ant species. Overall, sex allocation shows a significant correlation with the extent of competitive interactions between relatives, with a mean effect size of $r = 0.501$ (Table 1). Hence, competitive interactions among relatives explain 25.1% of the variance in sex allocation. This did not depend on the group of study—the mean effect size of the LRC ($r = 0.496$) and the mean effect size of the LMC ($r = 0.473$) were not significantly different (randomized ANOVA, $n = 9$, $P = 0.92$).

The effect sizes were highly variable but seemed to be uniformly distributed and showed no sign of publication bias. The trim and fill analysis did not detect missing studies ($n = 10$, number of missing studies $L_0 = 0$), we did not detect a lack

of studies with both nonsignificant result and small sample size (Spearman rank correlation test, $n = 10$, $r_s = -0.195$, $P = 0.59$), and the fail-safe number was large and above the quantitative criterion ($X = 155$, quantitative criterion $= 60$), which strongly suggests that there was no significant publication bias.

There was no significant difference between the mean effect sizes of competitive interactions, relatedness asymmetry variation, and queen number variation (randomized ANOVA, $n = 32$, $P = 0.15$).

DISCUSSION

Overall, this meta-analysis reveals that workers of social Hymenoptera bias colony sex allocation in their favor when relatedness asymmetry varies among colonies, as predicted by split sex ratio theory. When the queen was replaced by a daughter or mated with more than one male, variation in relatedness asymmetry explained 20.9% of the variance in sex allocation among colonies. This value is considerably higher than the average (3.6%) amount of variance explained in ecological and evolutionary studies (Møller and Jennions 2002) and hence provides a quantification of the success of split sex ratio theory.

While confirming the significant and pronounced impact of workers on colony sex allocation, the meta-analysis also reveals that worker control is far from complete as approximately 80% of the variance in sex allocation among colonies remains unexplained. Many uncontrolled stochastic factors can affect the sex ratio of field colonies, and a large amount of the variance in the data set might be due to such noise. However, part of the variance might also come from yet unrecognized adaptive predictors of sex allocation. One such potential source of variation among colonies that deserves further investigation is that queens might prevent worker manipulation by providing male-destined haploid eggs in some colonies and female-destined diploid eggs in other colonies (Passera et al. 2001; Roisin and Aron 2003; de Menten, Fournier, et al. 2005b; Rosset and Chapuisat 2006).

A somewhat surprising result of our survey was that very few studies contained quantitative data on both sex allocation and relatedness asymmetry variation. This contrasts with recent reviews that listed numerous qualitative results (Queller and Strassmann 1998; Bourke 2005) and suggests that further studies documenting quantitative variation are still needed to permit a more detailed analysis of the factors influencing the degree of sex ratio adjustment by workers.

One important aspect of the meta-analysis is that despite the small sample size, there was little sign of publication bias. In particular, there was no lack of studies on relatedness asymmetry variation that had small sample sizes and small effect sizes. Hence, the conclusion that workers bias colony sex allocation in their favor is unlikely to be due to the nonpublication of studies with negative results.

Queen number variation explained 5% of the variance, a value that is also significantly greater than 0. This result is consistent with worker control as relatedness asymmetry generally decreases when queen number increases. It is, however, difficult to evaluate the precise degree of adaptive sex allocation manipulation by workers because queen number variation is a variable of a different order that generally correlates with variation not only in relatedness asymmetry but also in LRC, life histories, and ecological factors (Ross and Keller 1995; Chapuisat and Keller 1999; Rosset and Chapuisat 2007).

The degree of worker control did not differ significantly among studies with different sources of variation in relatedness asymmetry (queen replacement, mate number, queen number). Hence, we found no support for the hypothesis that workers have more control when relatedness asymmetry is easier to assess (e.g., queen replacement versus mate number). Similarly, the degree of sex ratio adjustment by workers did not correlate significantly with the magnitude of the variation in relatedness asymmetry among colonies, suggesting that workers were not more likely to bias the sex ratio in their favor when differences in relatedness asymmetry among colonies were large. This contrasts with several previous analyses which have suggested that the strength of selection and information constraints may limit the extent of sex allocation adjustment in both vertebrates and invertebrates (Herre 1987; West and Sheldon 2002; Boomsma et al. 2003; Schino 2004; Sheldon and West 2004; Griffin et al. 2005). However, both analyses in this study suffer from a lack of power due to the very small and heterogeneous data set, and there were trends in the predicted direction, which stresses the importance of obtaining data from a greater range of species.

Overall, split sex ratio theory explained approximately 20% of the variance in sex allocation among social Hymenoptera colonies exhibiting variation in relatedness asymmetry, which is close to the 25% explained by LMC and LRC in the few species of social Hymenoptera where relatives compete. These values are very high compared to many ecological and evolutionary studies and confirm the remarkable predictive power of sex allocation theory (West et al. 2005). Moreover, worker control of sex ratio, LMC, and LRC are 3 processes based on kin selection. Hence, kin selection proves central and very successful at explaining sex allocation variation in social animals.

APPENDIX

Studies used in the meta-analysis. The "factor" indicates the source of variation being tested in each class of study, the "effect size" is the correlation between colony sex allocation and the relevant factor, and the "magnitude of relatedness asymmetry variation" is a measure of the proportion of variation in relatedness asymmetry among colonies in the population, estimated as $\frac{RA_{high}}{RA_{high} + RA_{low}}$ in which RA_{high} and RA_{low} are mean relatedness asymmetries in the highest and lowest relatedness asymmetry classes, respectively.

Species	Factor	Effect size (r)	Magnitude of relatedness asymmetry variation	Sample size	Reference
(a) Studies on relatedness asymmetry variation					
Ants					
Formica exsecta	Mate number	0.520[1]	0.60	42	Sundström et al. (1996)
Formica truncorum	Mate number	0.745[2]	0.60	17	Sundström and Ratnieks (1998)
Lasius niger	Mate number	−0.047[3]	0.55	64	Fjerdingstad et al. (2002)
Bees					
Augochlorella striata	Queen replacement	0.440[4]	0.75	38	Mueller (1991)
Bombus hypnorum	Mate number	0.081[5]	0.56	11	Paxton et al. (2001)
Halictus rubicundus	Queen replacement	0.786[6]	0.75	47	Yanega (1989), Boomsma (1991)
Lasioglossum laevissimum	Queen replacement	0.300[7]	NA	36	Packer and Owen (1994)
(b) Studies on queen number variation					
Ants					
F. exsecta	Queen number inferred from relatedness	0.270[8]	NA	41	Kümmerli R, personal communication
F. exsecta	Count of queens in polygyne colonies	0.476[9]	0.52	59	Brown and Keller (2000)
F. exsecta (mean of the 2 above studies)		0.386		50	
Formica podzolica	Monogyne versus polygyne colonies	0.180[10]	NA	84	Deslippe and Savolainen (1995)
Formica selysi	Monogyne versus polygyne colonies	0.008[11]	0.67	65	Rosset and Chapuisat (2006)
Lepthotorax acervorum	Monogyne versus polygyne colonies	0.411[12]	0.63	116	Hammond et al. (2002)
L. acervorum	Monogyne versus polygyne colonies	0.068[13]	NA	80	Chan et al. (1999)
L. acervorum	Monogyne versus polygyne colonies	0.065[14]	0.60	51	Heinze et al. (2001)
L. acervorum (mean of the 3 above studies)		0.200		247	
Leptothorax longispinosus	Monogyne versus polygyne colonies	0.278[15]	NA	103	Herbers (1984)
Myrmica ruginodis	Monogyne versus polygyne colonies	0.569[16]	0.68	45	Walin and Seppä (2001)
M. ruginodis	Count of queens in polygyne colonies	0.434[17]	NA	20	Walin and Seppä (2001)
M. ruginodis (mean of the 2 above studies)		0.533		33	
Myrmica tahoensis	Monogyne versus polygyne colonies	0.159[18]	NA	111	Evans (1995)
Pheidole pallidula	Monogyne versus polygyne colonies	−0.856[19]	0.45	26	Fournier et al. (2003)
P. pallidula	Count of queens in polygyne colonies	0.190[20]	NA	18	Helms et al. (2004)
P. pallidula (mean of the 2 above studies)		−0.501		22	
Stenamma debile	Monogyne versus polygyne colonies	0.101[21]	NA	57	Buschinger and Heinze (2001)
S. debile	Count of queens in polygyne colonies	0.037[22]	NA	40	Buschinger and Heinze (2001)
S. debile (mean of the 2 above studies)		0.075		49	
Wasps					
Brachygastra mellifica	Queen number inferred from relatedness	0.744[23]	0.55	12	Hastings et al. (1998)
Parachartergus colobopterus	Queen number inferred from relatedness	0.377[24]	0.65	19	Queller et al. (1993)
Polistes fuscatus	Monogyne versus polygyne colonies	0.294[25]	NA	17	Noonan (1978)
Polybia emaciata	Queen number inferred from relatedness	0.368[24]	0.61	20	Queller et al. (1993)
Polybia occidentalis	Queen number inferred from relatedness	0.317[24]	0.65	27	Queller et al. (1993)
Protopolybia exigua	Queen number inferred from relatedness	0.305[24]	0.59	29	Queller et al. (1993)
(c) Recent studies on competitive interactions among relatives that complement the data set of West et al. (2005)					
Ants					
Cardiocondyla minutior	LMC	0.225[26]		37	Heinze et al. (2004)
Cardiocondyla obscurior	LMC	0.593[27]		14	de Menten, Cremer, et al. (2005)
Cataglyphis cursor	LRC	0.089[28]		14	Pearcy and Aron (2006)

Notes on the calculation of effect sizes and magnitudes of relatedness asymmetry variation in Appendix (tables refer to each article).

[1] Proportion of females produced by single-mated queen versus multiple-mated queen colonies, $P_{1994} = 0.006$, $P_{1995} = 0.0001$. The relatedness asymmetry was inferred to be 3:1 and 2:1 in single-mated queen and multiple-mated queen colonies, respectively.

[2] Proportion of females produced by single-mated queen versus multiple-mated queen colonies, $F_{1,14} = 12.7$ for 1989–1991 and $F_{1,16} = 25.9$ for 1992–1995. The relatedness asymmetry was inferred to be 3:1 and 2:1 in single-mated queen and multiple-mated queen colonies, respectively.

[3] Sex investment ratio in single-mated queen versus multiple-mated queen colonies, Lausanne $Z_{(1997)} = -0.25$ and $Z_{(1998)} = -1.16$, Uppsala $Z = -0.19$. We calculated a weighted average from the relatedness asymmetries in Table 5, which were measured with 2 microsatellite markers.

[4] Sex investment ratio in eusocial (queen present) versus parasocial (queen replaced by a daughter) colonies, $t = 2.08$. The relatedness asymmetry was inferred to be 3:1 and 1:1 in eusocial and parasocial colonies, respectively.

Appendix footnotes, continued

[5] Sex investment ratio in single-mated queen versus multiple-mated queen colonies, data from Table 2, excluding the 3 colonies that produced fewer than 5 individuals, 1-tailed Wilcoxon rank-sum test, $P = 0.394$. The relatedness asymmetry, measured with 4 microsatellite markers, was 3:1 and 2.37:1 for single-mated queen and multiple-mated queen colonies, respectively.

[6] Proportion of females produced by eusocial (queen present) versus parasocial (queen replaced by a daughter) colonies, chi-squared test, $\chi^2 = 29.02$. The relatedness asymmetry was inferred to be 3:1 and 1:1 in eusocial and parasocial colonies, respectively.

[7] Correlation between sex investment ratio and relatedness asymmetry, $r_s = 0.30$. The relatedness values between classes of nest mates indicate that queen replacement is the most likely source of relatedness asymmetry variation among colonies.

[8] Correlation between sex investment ratio (proportion or resources allocated to females) and worker-brood relatedness in polygyne colonies, unpublished data, $r_s = 0.270$.

[9] Genetic effective queen number in female- versus male-producing polygyne colonies, $F_{1,39} = 11.43$. We calculated the relatedness asymmetry in polygyne colonies as described in Methods, using effective queen number of 2.7 and worker relatedness of 0.087 in female-producing colonies and effective queen number of 6.7 and worker relatedness of 0.062 in male-producing colonies.

[10] Proportion of males produced by polygyne versus monogyne colonies, $F_{2,78} = 1.16$, $P = 0.03$. The OH test on these statistics gave $r_s = 1$, $P_c = 0.70$, and final $P = 0.05$.

[11] Sex investment ratio in monogyne versus polygyne colonies, $Z_{(2001)} = -0.11$ and $Z_{(2002)} = -0.01$. The relatedness asymmetry, measured with 9 microsatellite markers, was 2.76:1 and 1.39:1 in monogyne and polygyne colonies, respectively.

[12] Sex investment ratio in monogyne versus polygyne colonies, $F_{1,110} = 22.3$. The relatedness asymmetry, measured with 5 microsatellite markers, was 3.4:1 and 2.0:1 for monogyne and polygyne colonies, respectively.

[13] Sex investment ratio in monogyne versus polygyne colonies, excluding data from the Santon population which were presented in Hammond et al. (2002), Aberfoyle $F_{1,29} = 0.02$ and Roydon $F_{1,23} = 0.39$.

[14] Sex investment ratio in monogyne versus polygyne colonies, $P = 0.64$. The relatedness asymmetry in monogyne colonies was inferred to be 3:1. We calculated the relatedness asymmetry in polygyne colonies as described in Methods, using the median queen number of 3 and worker relatedness of 0.493.

[15] Proportion of males produced by polygyne versus monogyne colonies, $P = 0.07$. The OH test on these statistics gave $r_s = 1$, $P_c = 0.93$, and final $P = 0.0024$.

[16] Sex investment ratio in monogyne versus polygyne colonies, Leimann $P = 0.031$ and Täktom $P = 0.002$. The relatedness asymmetry was measured with 4 allozyme loci. In the Leimann population, it was 2.71:1 and 1.55:1 for monogyne and polygyne colonies, respectively. In the Täktom population, it was 2.68:1 and 0.92:1 for monogyne and polygyne colonies, respectively.

[17] Correlation between sex investment ratio and queen number in polygyne colonies, from the data in Appendix, Leimann $r_s = -0.457$ and Täktom $r_s = -0.395$.

[18] Sex investment ratio in queenless monogyne and polygyne colonies, $\chi^2_{(2)} = 2.79$. The data set was not amenable to OH test, so the effect size for monogyne versus polygyne colonies is conservative.

[19] Sex investment ratio in monogyne versus polygyne colonies, $F_{1,22} = 60.13$. The relatedness asymmetry, measured with 4 microsatellite markers, was 2.66:1 and 3.32:1 for monogyne and polygyne colonies, respectively. These values do not differ significantly, and the magnitude of relatedness asymmetry variation was estimated with the relatedness asymmetry of monogyne colonies in the numerator.

[20] Correlation between proportion of females and queen number in polygyne colonies, $r_s = -0.19$.

[21] Proportion of females produced by monogyne versus polygyne colonies, data from Tables 2–4, excluding queenless colonies, 1-tailed Wilcoxon rank-sum test, $W = 369$, $P = 0.223$.

[22] Correlation between sex investment ratio and queen number in polygyne colonies, data from Tables 2–4, excluding queenless colonies, Spearman rank correlation test, $r_s = -0.037$.

[23] Relatedness among females in female-producing colonies versus relatedness among workers in male-producing colonies, 1-tailed t-test, $P < 0.005$. The effect size is conservative because it had to be calculated from the P value threshold. We calculated the relatedness asymmetry as described in Methods, estimating queen number from worker relatedness given in Table 1.

[24] Relatedness among females in female-producing colonies versus relatedness among workers in male-producing colonies, t-test, $P < 0.05$ for each of the 4 species. Effect sizes are conservative because they had to be calculated from the P value thresholds. We calculated the relatedness asymmetry as described in Methods, using relatedness among queens and relatedness among workers in female- and male-producing nests, respectively.

[25] Sex investment ratio in colonies founded by one versus several queens, data from Table 1, Wilcoxon rank-sum test, $W = 41.5$, $P = 0.113$.

[26] Proportion of haploid eggs in polygyne versus monogyne colonies, $\chi^2_{(4)} = 2.161$. The OH test on these statistics gave $P = 0.086$.

[27] Proportion of ergatoid males in polygyne versus monogyne colonies, $\chi^2 = 4.93$.

[28] Correlation between proportional investment in females and total sexual productivity, $Z = -0.334$.

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When it is costly to have a caring mother: food limitation erases the benefits of parental care in earwigs

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Animal behaviour

When it is costly to have a caring mother: food limitation erases the benefits of parental care in earwigs

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The aggregation of parents with offspring is generally associated with different forms of care that improve offspring survival at potential costs to parents. Under poor environments, the limited amount of resources available can increase the level of competition among family members and consequently lead to adaptive changes in parental investment. However, it remains unclear as to what extent such changes modify offspring fitness, particularly when offspring can survive without parents such as in the European earwig, *Forficula auricularia*. Here, we show that under food restriction, earwig maternal presence decreased offspring survival until adulthood by 43 per cent. This effect was independent of sibling competition and was expressed after separation from the female, indicating lasting detrimental effects. The reduced benefits of maternal presence on offspring survival were not associated with higher investment in future reproduction, suggesting a condition-dependent effect of food restriction on mothers and local mother–offspring competition for food. Overall, these findings demonstrate for the first time a long-term negative effect of maternal presence on offspring survival in a species with maternal care, and highlight the importance of food availability in the early evolution of family life.

Keywords: family life; conflicts; food restriction

1. INTRODUCTION

Parental care has been described in a variety of species across the animal kingdom, wherein one or two parents attend their brood. Parental presence generally increases offspring survival through protection against predators and pathogens or through food provisioning. But such parental care often comes at costs to parents (see [1,2] for a review). For instance, it may reduce their capability to invest in future reproduction, delay successive broods or decrease their likelihood to survive until the next reproductive period [3,4]. To maximize fitness returns on investment, parents are expected to adjust their level of care in relation to variation in benefits to offspring and in costs to themselves [5]. Hence, understanding which factors affect benefits and costs of parental care provides key insight into the conditions under which parental care evolved [6].

Whereas a growing number of studies have investigated how genetic conflicts and variation in relatedness among family members influence the costs/benefits ratio of parental care [2,7], little is known about the effects of external environmental conditions and their influence on the early evolution of parental care [2]. A traditional hypothesis is that harsh environments favour the evolution of parental care [8] owing to the enhanced benefits to offspring survival [1,2]. However, such environments are also expected to exacerbate the cost of care and reduce the value of current offspring for parents, who may consequently favour investments in future reproduction to the detriment of current offspring [5,9,10]. In line with this idea, positive associations between resource availability and parental care have been reported in a few altricial species where offspring cannot survive without care (reviewed in [1]). While such patterns provide information about when parents should stop caring owing to enhanced costs, these studies are of limited relevance to understand how environmental quality shapes the early evolution of parental care and family life [4,11,12].

Here, we tested whether maternal presence under restricted food conditions influenced offspring survival and female investment in future reproduction in the European earwig, *Forficula auricularia*. This species is ideal for our question since mothers protect their clutches against natural enemies, provide food to their young and their presence improves offspring (nymph) survival under ad libitum food conditions. Furthermore, nymphs can survive and feed for themselves in the absence of mothers, and females can lay a second and final clutch a few weeks after having tended first clutch nymphs [4,13,14]. To assess whether maternal presence possibly decreases offspring survival by exacerbating the effects of sibling competition (partly mediated by sibling cannibalism [15]), we also manipulated brood size [4] and siblings' relatedness [15] in addition to maternal presence.

2. MATERIAL AND METHODS

We set up a total of 132 experimental groups of nymphs under restricted food availability, in which we manipulated the presence of mothers, the number of nymphs (brood size) and their relatedness. Maternal presence and brood size were manipulated by splitting 44 clutches of one-day-old nymphs (= clutch of origin) into three experimental groups: two groups of 10 (small group) and 20 (large group) nymphs without mother, and one group of either 10 or 20 nymphs with their own mother (see the electronic supplementary material, table S1). Nymph relatedness was manipulated by controlling the number of mating partners their mothers previously had access to: 23 females were singly mated and 21 females were multiply mated each with a different male (i.e. successively mated with four unrelated males) [13]. Adult earwigs used in this experiment were the first laboratory-born generation produced by individuals sampled in May 2009 in Dolcedo, Italy.

At set up, the 132 groups were maintained in small Petri dishes for 16 days, which corresponds to the approximate duration of family life in earwigs [14]. On day 16, we determined the proportion of nymphs still alive and the proportion of surviving nymphs that had reached the second juvenile instar (= developmental rate). All nymphs were subsequently transferred to medium Petri dishes for 15 days, and then to large Petri dishes from which the proportion of nymphs surviving until adulthood was later determined. Simultaneously, tending mothers were isolated in medium Petri dishes on day 16 to quantify the occurrence and the size of second clutches [13]. The fresh weights of mothers were measured at egg hatching and on day 16.

To ensure food restriction, we provided each group with a standardized amount of food every six days (diet composition in [13]) and removed the left-over food three days after supply (food was generally

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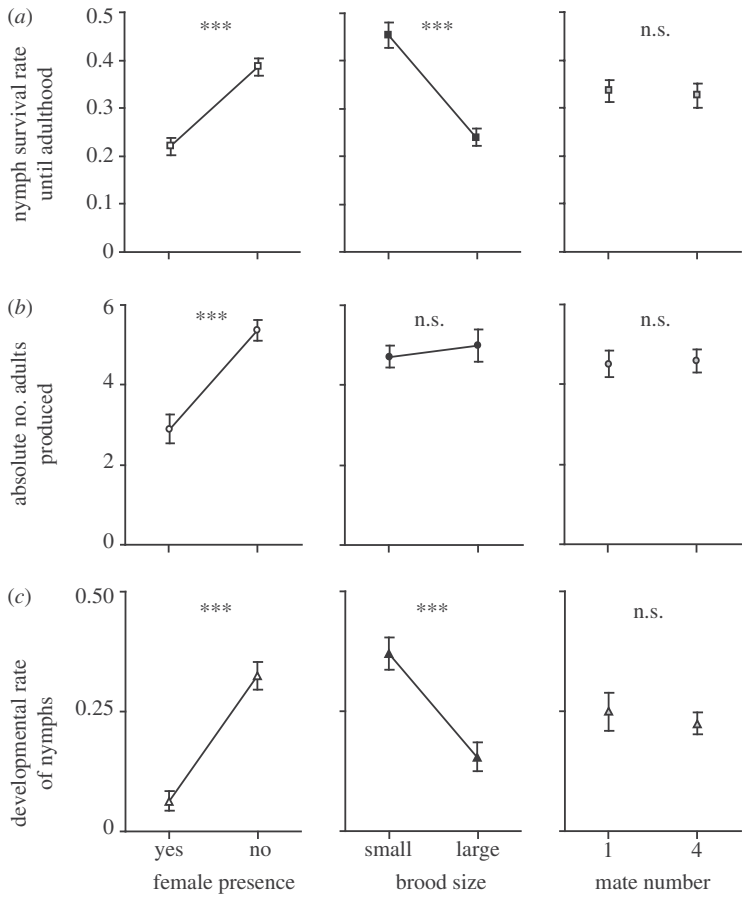

Figure 1. Effects of maternal presence, brood size and mate number on (*a*) nymph survival rate until adulthood, (*b*) absolute number of adults and (*c*) nymph developmental rate. Paired-values (lines) come from split clutches. ***$p < 0.0001$, $^{n.s.}p > 0.70$.

finished in two days). The quantity of food was adjusted according to the age of the nymphs, with 60, 120 and 240 mg while in small, medium and large Petri dishes, respectively. Females had access to ad libitum food once isolated for second clutch production [13].

Survival and developmental rates were tested using general linear mixed models (GLMMs) with binomial error distributions, while the absolute number of emerging adults was tested using GLMMs with a Gaussian error distribution. Each model was computed using maternal presence, brood size and mating treatment as fixed factors, and the clutch of origin as the random factor. To assess whether variation in the benefits of maternal presence (BMP) on offspring survival until adulthood was related to female food intake and future reproduction, analyses of covariance were used with BMP as a covariate, brood size and mating treatment as fixed factors, and either female relative weight gain on day 16, number of second clutch eggs produced or relative investment of females into second clutch (= number of second clutch eggs divided by the number of total eggs [13]) as the response variable. BMP was calculated by subtracting the survival rates of nymphs without mothers from the ones with mothers, between groups of same size and same clutch of origin. All statistical models were conducted using R v. 2.14.0.

3. RESULTS

The survival rate of nymphs until adulthood was significantly lower in groups tended by mothers (figure 1*a*; $t = -5.53$, d.f. $= 86$, $p < 0.0001$) and in large groups ($t = -7.82$, d.f. $= 86$, $p < 0.0001$). The above effects on survival rate until adulthood translated into a smaller number of adult produced in groups tended by mothers (figure 1*b*, $t = -6.21$, d.f. $= 86$, $p < 0.0001$) but not in large groups ($t = -0.33$, d.f. $= 86$, $p = 0.75$). Mating treatment neither significantly influenced the survival rate of nymphs until adulthood (figure 1*a*, $t = -0.18$, d.f. $= 42$, $p = 0.86$) nor adult numbers (figure 1*b*, $t = 0.21$, d.f. $= 42$, $p = 0.83$). No interaction significantly influenced the two above traits (all $p > 0.26$).

The survival rate of nymphs until day 16 (i.e. when tending mothers were removed) was not significantly influenced by maternal presence ($t = -1.34$, d.f. $= 86$, $p = 0.19$), brood size ($t = 0.81$, d.f. $= 86$, $p = 0.42$), mating treatment ($t = 0.96$, d.f. $= 42$, $p = 0.35$) nor by any interaction (all $p > 0.27$). By contrast, the developmental rate of nymphs measured at day 16 was lower both in presence of mothers (figure 1*c*; $t = 9.08$, d.f. $= 86$, $p < 0.0001$) and in large groups ($t = -10.14$, d.f. $= 86$, $p < 0.0001$). There was no significant effect of the mating treatment on developmental rate (figure 1*c*; $t = 0.39$, d.f. $= 42$, $p = 0.70$) and none of the tested interactions was significant (all $p > 0.70$).

BMP was significantly and negatively correlated with the female weight gain until day 16 (figure 2; $r = -0.36$; likelihood ratio, $\chi^2 = 6.23$, $p = 0.013$; other factors and interactions: all $p > 0.15$). However, BMP did not predict maternal investment in future reproduction, as it was not significantly associated with the number of second clutch eggs ($r = 0.08$, LR, $\chi^2 = 0.29$, $p = 0.59$; other effects and interactions: all $p > 0.10$) or with the relative investment of females in second clutch ($r = 0.14$, LR, $\chi^2 = 0.81$, $p = 0.37$; other effects and interactions: all $p > 0.09$).

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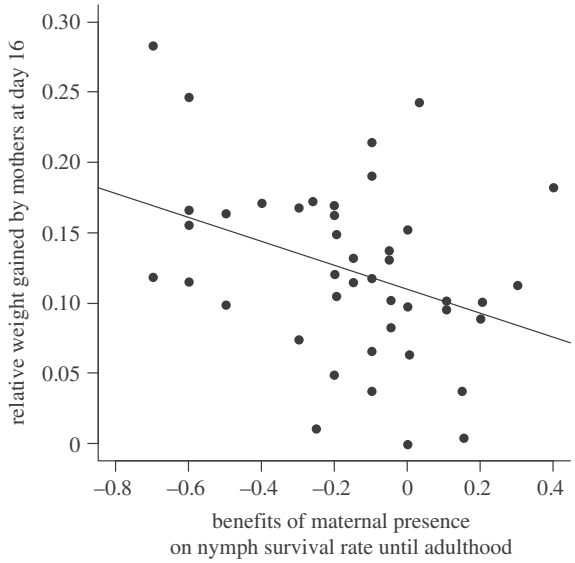

Figure 2. Benefits of maternal presence (BMP) and relative weight gained by mothers during family life.

4. DISCUSSION

Models for the evolution of parental care and family life generally assume that parents pay a fitness cost for tending their brood, whereas their presence provides fitness benefits to offspring [5,6]. Here, we demonstrate that under food restriction, the usual BMP for offspring (approx. 15% increase in the survival rate [4]) are erased: females reduced offspring survival by 43 per cent. This result is at odds with the traditional hypothesis that harsh environments should favour the evolution of parental care [8]. Furthermore, the above effect emerged after the period of family life, which excludes the immediate influence of females on offspring survival (e.g. through filial cannibalism [10]), but emphasizes their critical action on developmental processes affecting long-term survival. This interpretation is further supported by the negative effect of maternal presence observed on nymph developmental rate.

Parents are predicted to adaptively reduce offspring numbers when (i) it limits sibling competition over resources and consequently improves the survivorship of the remaining offspring, and/or when (ii) it allows parents to invest the resulting gain of energy into future reproduction [1,10,16,17]. Our results support neither of these two hypotheses. First, the presence of mothers did not modify the level of sibling competition, as there was no interaction effect between maternal presence and brood size on nymph survival. Secondly, we found that BMP reduction, albeit associated with an increased gain of weight in females, did not translate into larger maternal investment in second clutches. One reason for the latter result may be that the ad libitum access to food after day 16 in the experiment allowed females to invest in future reproduction without being strongly affected by earlier food restriction. Another non-mutually exclusive explanation could be that BMP reflects a condition-dependent effect of food restriction on females, where those exhibiting low intrinsic condition compete more intensely with their offspring for food. In line with this hypothesis, we found considerable variation in how maternal presence influenced the offspring survival rate (figure 2), with nine females (20%) enhancing offspring survival despite food restriction.

Sibling competition influenced offspring survival independently of maternal presence, a result not fully in line with burying beetles where maternal presence exacerbates sibling competition [16]. Living in large broods reduced survival rate of nymphs until adulthood but did not affect the absolute number of adults produced. Because all groups had access to a similar amount of food, this finding suggests a negative association between *per capita* food availability and sibling cannibalism in earwig families [15]. Conversely, variation in sibling relatedness had no significant effects on nymph survival. Provided that multiple mating translated into multiple paternity, this result suggests that preferential siblicide between nymphs from different clutches [15] does not reflect genetic kin recognition mediated by competition between siblings from different patrilines [17].

To conclude, this study demonstrates that harsh environments erase the benefits of parental care in a species with facultative care, possibly through mother–offspring competition for the limited resources. In such species, and at an early evolutionary stage, resource availability therefore affects the nature and extent of selection on parents versus offspring and can generate conditions where parental presence become detrimental to offspring fitness and parental care not adaptive.

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Paternal signature in kin recognition cues of a social insect: concealed in juveniles, revealed in adults

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Paternal signature in kin recognition cues of a social insect: concealed in juveniles, revealed in adults

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Kin recognition is a key mechanism to direct social behaviours towards related individuals or avoid inbreeding depression. In insects, recognition is generally mediated by cuticular hydrocarbon (CHC) compounds, which are partly inherited from parents. However, in social insects, potential nepotistic conflicts between group members from different patrilines are predicted to select against the expression of patriline-specific signatures in CHC profiles. Whereas this key prediction in the evolution of insect signalling received empirical support in eusocial insects, it remains unclear whether it can be generalized beyond eusociality to less-derived forms of social life. Here, we addressed this issue by manipulating the number of fathers siring clutches tended by females of the European earwig, *Forficula auricularia*, analysing the CHC profiles of the resulting juvenile and adult offspring, and using discriminant analysis to estimate the information content of CHC with respect to the maternal and paternal origin of individuals. As predicted, if paternally inherited cues are concealed during family life, increases in mating number had no effect on information content of CHC profiles among earwig juveniles, but significantly decreased the one among adult offspring. We suggest that age-dependent expression of patriline-specific cues evolved to limit the risks of nepotism as family-living juveniles and favour sibling-mating avoidance as group-living adults. These results highlight the role of parental care and social life in the evolution of chemical communication and recognition cues.

1. Introduction

The evolution of group living selects for recognition mechanisms ensuring that cooperative and aggressive behaviours are directed towards the appropriate individuals, but also that adult group members avoid the costs of sibling-mating. In insects, information about encountered individuals is typically displayed by the chemical cues present on the waxy layer covering their cuticle: the cuticular hydrocarbons (CHCs) [1–3]. CHC profiles have been shown to reflect information about different aspects of an individual's identity, such as the species [4] or the sex [5]. Inter-individual variation in CHC profiles is common in nature and typically due to various not mutually exclusive sources. For instance, CHC profiles have been shown to change over the course of an individual's life cycle, e.g. owing to aging [6] or to changes in individual tasks within colonies of eusocial insects [7], they can be influenced by the environment, such as the nesting substrate [8,9], nutritional condition [10,11] or social interactions with conspecifics, which mediates the active or passive transfer of chemical compounds between individuals [12–14]. Finally, CHC profiles can also vary owing to genetic differences between individuals (e.g. [15,16]). A heritable component to variation in CHCs is important for long-term similarities of CHC profiles among individuals originating from the same family or colony and thus, for CHCs to represent informative and sufficiently stable cues for individual identity and kin recognition (e.g. [3,17]).

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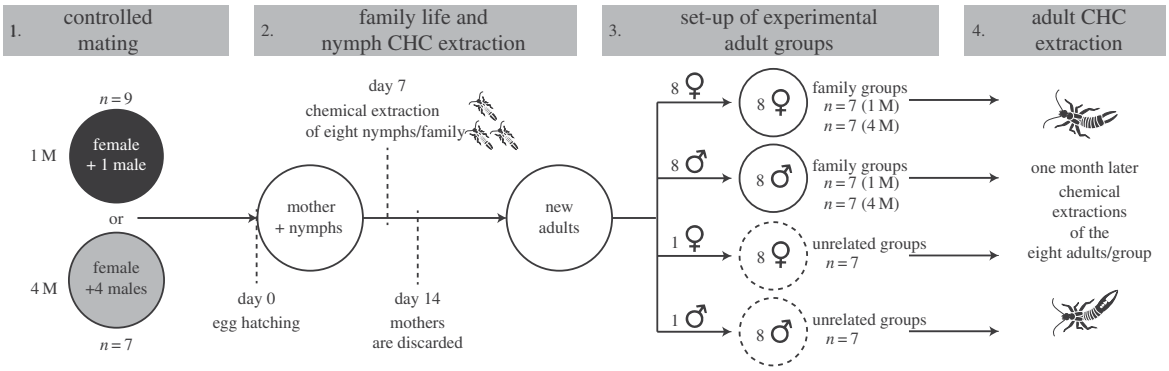

Figure 1. Experimental design used to extract the CHC profiles from nymph and adult earwigs.

The importance of social conflicts on the expression of parent-of-origin specific cues in offspring has been a central and often controversial point in the research on the evolution of insect communication and social life (e.g. [18,19]). In particular, it has been suggested that polyandrous colonies of eusocial insects (ants, some bees and wasps) select against the expression of patriline-specific signals in their offspring (e.g. eggs, larvae and workers), because the expression of such signals could enhance the risks of paternally driven nepotistic conflicts between colony members and thus ultimately reduce colony efficiency and the fitness of group members [18,20]. Whereas this prediction received empirical support in several Hymenoptera species (e.g. reviewed in [21]; but see [22–24]), it remains unclear whether this process is specific to the derived eusocial systems or a more widespread phenomenon involved in the early evolution of social life. Disentangling these issues therefore requires investigating the occurrence of such a mechanism in insect species expressing non-derived forms of social life, such as the ones with temporary family life and maternal care. In families with uniparental female care and where multiple paternity occurs within a female's clutch, paternally inherited genes are predicted to select for the expression of patriline-specific signals in their offspring, which would favour cooperation between their own descendants and competition against the paternally unrelated half-siblings. Conversely, sibling competition generally reduces the number and/or quality of a mother's offspring [25], so that tending females (and/or the maternally inherited genes) could benefit from limiting the expression of patriline-specific cues in the offspring. Importantly under this hypothesis, the maternal concealment of information about the paternal origin of offspring should be limited to juveniles. When offspring become reproductive adults, they could otherwise suffer from the concealment of cues that are possibly used to limit the risks of inbreeding depression. Although this age-specific expression of patriline-specific cues in offspring is of key importance to better understand the joint evolution of insect communication and social life, its occurrence remained surprisingly unexplored so far.

In this study, we investigated whether variation in CHC profiles contains information allowing kin recognition in the European earwig *Forficula auricularia* L. (Dermaptera: Forficulidae), and whether the profiles are associated with an age-specific expression of paternally inherited cues in offspring. In this species, clutches are often sired by multiple males [26,27]. The offspring (nymphs) live in family groups for several weeks, during which females provide multiple forms of care, such as egg and offspring attendance and food provisioning [27,28]. Previous work has shown that sibling competition and cannibalism are common in this species [29] and occurs significantly earlier and more often between unrelated nymphs from different clutches [29]. Thus, kin recognition cues seem to be present and used, and cannibalism is a potential form of nepotistic interactions among young nymphs. Once adult, *F. auricularia* individuals live in mixed-sex groups [27]. Inbreeding (sibling-mating) was shown to entail substantial fitness costs in this species [30], which could have thus selected for the expression of maternally and/or paternally inherited recognition cues to allow individuals avoiding mating with close relatives and thus limiting inbreeding depression.

We addressed the four following questions to test the predictions on the information content of CHC profiles in *F. auricularia*. (i) Is variation in CHC profiles smaller among individuals from the same than from different families, as expected if chemical signatures are family specific? (ii) Is within family variation larger in broods sired by multiple males compared with broods sired by a single male, as expected if the cues are heritable and display a signature of paternal origin? (iii) Is the expression of a paternal signature, i.e. higher variation in CHC profiles among offspring in multiply sired clutches, absent in offspring, but present in adults, as predicted under age-dependent concealment of paternally inherited cues? Finally, (iv) does the environment shared by adults also contribute to variation in their chemical profiles and thus possibly hamper family recognition after family disruption?

2. Material and methods

(a) Experimental design

The chemical signatures of 112 nymphs and 329 adults of *F. auricularia* were extracted from 16 experimental clutches (figure 1). These clutches were second clutches of either nine females mated to a single male ('singly mated females' = 1 M-treatment) or seven females mated to four successive unrelated males (each male was used only once across all the mating trials; 'multiply mated females' = 4 M-treatment). The 16 mothers (and their mates) were from a second laboratory-born generation of individuals sampled in May 2009 in Dolcedo (Italy). They were reared under standardized laboratory conditions until each female produced her first clutch (see details in [31]). Sixteen days after their first clutch hatched, all 16 females were isolated individually in a small Petri dish (10 cm diameter) for a second clutch production. The Petri dishes were kept in a

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climate chamber at 15°C, 60% humidity and complete darkness until egg laying and hatching.

One day after hatching of their second clutch (day 1), each mother and 37.6 ± 1.28 nymphs (mean $\pm$ s.e.) of her second clutch were transferred into new Petri dishes and subsequently reared at 20°C, 60% humidity and 14 L : 10 D cycle. On day seven, eight nymphs per clutch (from seven 1 M and seven 4 M clutches) were randomly sampled, singly isolated in glass-vials (300 µl) and immediately frozen at −20°C for later chemical extractions. The remaining nymphs were kept with their mothers until day 14. Then mothers were removed and all nymphs were transferred to large Petri dishes (14 cm diameter) until their adulthood [31]. Just after moulting into adults, males and females of each family were separated in two new large Petri dishes to prevent sibling-mating. Once all individuals became adults, eight males and eight females were randomly sampled in each family and set up in new large Petri dishes (called family groups) with seven 1 M groups and seven 4 M groups per sex. We used the same seven 4 M families to sample nymphs and adults. But owing to small clutch sizes, we used five 1 M families to sample both nymphs and adults, two 1 M families to sample only nymphs and two different 1 M families to sample only adults.

To test the influence of shared environment (i.e. the Petri dish) on variations in adult chemical profiles, we mixed adults from 12 experimental clutches (both 1 M and 4 M) to form seven groups of eight unrelated females and seven groups of eight unrelated males (called unrelated groups, figure 1). One month later, all adults were frozen during 2 h at −20°C, then individually transferred to a 2 ml glass vial and kept at −20°C until chemical extractions. Except when mentioned, all Petri dishes contained humid sand as a substrate, one plastic tube as shelter and received ad libitum food changed twice a week [31].

(b) Chemical extraction

CHCs from nymph, female and male were extracted individually for 10 min using 60 µl (nymphs) or 800 µl (adults) of *n*-Heptane (Carl-Roth AG, Arlesheim, Switzerland) as solvent, and *n*-Octadecane as internal standard (concentration of 2.5 ng μl^{-1}, Fluka Analytical, Sigma-Aldrich, Buchs, Switzerland). The extracts were subsequently analysed by gas chromatography-mass spectrometry. Full description of chemical analyses are provided in the electronic supplementary material.

(c) Statistical analyses

Chemical extraction resulted in a total of 19 peaks of CHCs in nymphs and 19 in adults. Peaks 18 (nymphs) and 25 (adults) were excluded from the analyses because peak 18 was collinear to peak 19 in nymphs (table 1; Pearson correlation, $t_{110} = 29.55$, $r = 0.94$, $p < 0.0001$) and peak 24 to peak 25 in adults (table 1; Pearson correlation; $t_{327} = 54.53$, $r = 0.95$, $p < 0.0001$), resulting in 18 peaks in adults and nymphs. The results remained unchanged when peaks 19 and 25 were excluded instead. We subsequently conducted a series of linear discriminant analyses (DA) to investigate the degree to which the chemical signature of nymphs and adults reflected their family of origin, and how this information content varied with the number of fathers that sired the clutch (table 1). The significance of each DA was evaluated both using Wilks' λ tests and prediction success (by estimating the percentage of correct assignment of individuals to their family of origin) through cross validation (leave-one-out method). The cross validation allowed us to control for potential overfitting of the data by the statistical models. We used 18 peaks for adults and 18 for nymphs, which corresponded to the recommendation in multivariate statistics like DA, that sample size should be at least three times the number of variables used [11]. To avoid limitations inherent to analyses of compositional data (as is the case for the CHC profiles), the area of each peak was transformed according to Aitchison formula [32] prior to DA (for details, see [13]). Comparable results were found when the DA were done on an estimation of the absolute quantity of each peak using a known internal standard.

We first tested the degree to which the chemical signature of nymphs and adults generally reflected their family of origin using two DA based on the chemical signatures of either all nymphs or all adults reared in family groups. We then analysed whether these DA remained significant when taking into account the mating treatment and the age (and the adult sex) of the tested individuals by conducting a series of six DA based on each combination of nymphs, males and females sampled in 1 M and 4 M groups (table 1). Finally, we tested whether the chemical signature of adults at least partly reflected their shared environment (i.e. the shared Petri dish) using two DA based on the chemical signatures of either males or females from unrelated groups.

The prediction successes obtained from the cross-validation method were compared using general linear models (GLMs) with binomial error distribution. To this end, the prediction success obtained from the cross-validation method on each DA was converted into a binomial vector (1 or 0 values) of a length equal to the number of individuals involved in the DA and wherein the proportion of 1 was equal to the prediction success obtained from the cross-validation method. The prediction successes of nymphs from 1 M and 4 M families was compared using a GLM wherein 1 M/4 M was entered as fixed factor, and the two binomial vectors reflecting the respective prediction success concatenated to form the response variable (we used the same process to generate the response variables in all the following GLMs). The prediction successes of adults from 1 M and 4 M families were then compared using a GLM wherein mating treatment, sex and their interaction were entered as fixed factors. Adult sex was included in the model to control for potential sex-specific CHC profiles in earwig adults. Finally, whether the chemical signature of adults at least partly reflects their shared environment was tested by comparing the prediction successes of adults between 1 M families and unrelated groups and the ones of adults between 4 M families and unrelated groups using two GLMs, in which the type of group (family versus unrelated), the sex of the adults and their interactions were entered as fixed factors. Adults from 1 M and 4 M groups were analysed separately because mating treatment influenced the prediction success of adults (see Results). All statistical analyses were conducted using the software R. 3.0.2 (http://www.r-project.org/).

3. Results

The 19 CHCs present on the cuticular extracts of individual earwigs did not only exhibit quantitative differences between life-stages (table 1), but also qualitative ones with eight CHCs specific to nymphs and eight to adults. Overall, the chemical signatures of nymphs and adults significantly predicted their family of origin (nymphs: Wilk's $\lambda < 0.0001$, approx. $f = 11.06$, $p < 0.0001$; adults: Wilk's $\lambda = 0.0001$, approx. $f = 4.70$, $p < 0.0001$). The prediction successes were 92.0% and 64.1% using cross validation for nymphs and adults, respectively. Interestingly, these predictions remained significant when taking into account the sex of the tested adults and/or the mating treatment (table 2 and figure 2, all $p < 0.0001$), with the corresponding successes ranging from 52.7 to 81.8% (figure 2).

As predicted under the age-dependent concealment of paternally inherited cues in offspring, the mating treatments did not affect variation in the nymph chemical profiles, but affected the ones in adult chemical profiles. The prediction success of nymphs was not significantly different between the mating treatments (figure 2; GLM, Likelihood ratio (LR) $\chi^2_1 = 0.44$, $p = 0.506$), but the one of adults was significantly higher in the 1

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Table 1. Mean relative peak area (%) of the CHCs extracted from 112 nymphs, 163 females and 166 males of the European earwig. (KI, mean Kovats retention index; IS, internal standard.)

	CHC	KI	males	females	nymphs
(1)	nC13	1300	—	—	7.24
(2)	nC15	1500	—	—	1.18
IS	nC18	1801	—	—	—
(3)	nC21	2100	—	—	4.77
(4)	X,X′-nC23 : 2 + X″-nC23 : 1	2275	—	—	10.68
(5)	X‴-nC23 : 1	2281	—	—	2.86
(6)	nC23	2300	0.14	0.15	9.33
(7)	11-, 9-MeC23	2334	—	—	1.15
(8)	5-MeC23	2348	—	—	0.68
(9)	3-MeC23 + (X-nC24 : 1)	2370	—	—	1.45
(10)	X,X′-nC25 : 2 + X″-nC25 : 1	2474	0.08	0.22	38.51
(11)	nC25	2501	1.37	2.20	3.84
(12)	13-, 11-, 9-MeC25	2533	1.03	2.60	2.77
(13)	3-MeC25 + (X-nC26 : 1)	2570	0.20	0.88	1.19
(14)	13-, 11-, 9-MeC26	2633	0.10	0.29	—
(15)	X,X′-nC27 : 2 + X″-nC27 : 1	2675	0.44	0.63	3.36
(16)	nC27	2701	1.62	1.58	0.37
(17)	13-, 11-, 9-, 7-MeC27	2743	25.91	35.94	2.99
(18)	7,15-; 7,19-; 11,15-; 11,17-; 11,19-diMeC27[a]	2769	6.87	14.33	0.58
(19)	2,17-; 2,19-; 2,21-; 2,23-diMeC27	2776	4.25	8.30	0.70
(20)	13-, 11-, 9-, 7-MeC28	2836	0.94	1.15	—
(21)	9,15-diMeC28	2865	1.30	1.76	—
(22)	13-, 11-, 9-, 7-MeC29	2943	24.46	11.38	0.47
(23)	7,19-; 9,19-; 11,17-; 11,19-diMeC29	2966	22.94	13.22	—
(24)	15-, 13-, 11-, 9-MeC30	3037	3.69	2.00	—
(25)	15-, 13-, 11-, 9-MeC31[b]	3138	1.58	0.39	—
(26)	7,19-; 9,19-; 9,21-diMeC31	3167	1.49	0.36	—
(27)	13-, 11-MeC33	3335	0.17	0.09	—

[a]Excluded from the DA on nymphs owing to collinearity.
[b]Excluded from the DA on adults owing to collinearity.

Table 2. Results of discrimination analyses of earwig individuals according to their life stage, their sex (only for adults), the type of rearing group (family or unrelated) and the mating treatment (1 M or 4 M). (The table indicates the number of families used (*N* fam) and the total number of individuals (*N* ind) per type of experimental group.)

life stage	rearing group	mating treatment	*N* fam	*N* ind	λ	approx. *f*-value	*p*-value
nymphs	family	1 M	7	56	<0.0001	11.05	<0.0001
	family	4 M	7	56	<0.0001	15.2	<0.0001
males	family	1 M	7	55	0.0002	5.69	<0.0001
	family	4 M	7	56	0.0003	4.87	<0.0001
	unrelated	—	7	55	0.0095	1.97	<0.0001
females	family	1 M	7	54	0.0009	3.67	<0.0001
	family	4 M	7	55	0.0033	2.67	<0.0001
	unrelated	—	7	54	0.0083	1.98	<0.0001

M compared with the 4 M groups (figure 2; GLM, LR $\chi^2_1 = 5.55$, $p = 0.018$). The prediction success of adults was also significantly higher among males than females (figure 2; GLM, LR $\chi^2_1 = 5.13$, $p = 0.024$), but not significantly influenced by an interaction between the mating treatments and the sexes of the adult individuals (GLM, LR $\chi^2_1 = 0.49$, $p = 0.485$).

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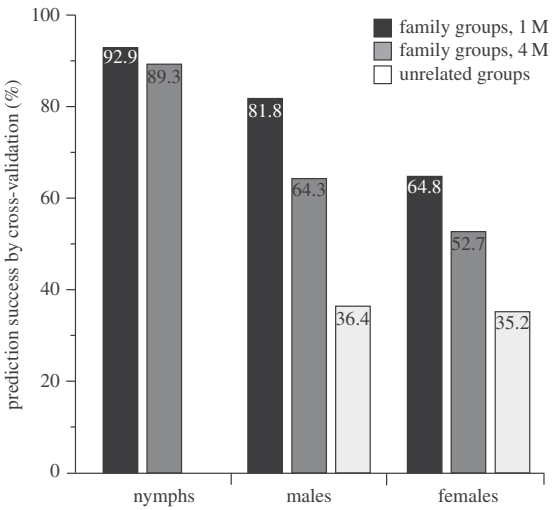

Figure 2. Prediction success by jack-knife cross validation of earwig individuals according to their life stage, their sexes (only for adults), the type of rearing groups (family or unrelated) and the mating treatments (1 M or 4 M). The corresponding values are given at the top of each bar.

Independently from the mating treatments, our results also showed that the chemical signature of adults partly reflected the environment in which they had been reared. The DA performed on the groups of unrelated adults significantly separated each sex according to their experimental groups (table 2 and figure 2), with 36.4% of males and 35.2% of females correctly assigned to their experimental groups by cross-validation method. Nevertheless, adult chemical profiles reflected more their clutch of origin than their groups/environments, as the prediction successes were lower among unrelated groups than 1 M family groups (GLM, group type: LR $\chi^2_1 = 18.19$, $p < 0.0001$; sex: LR $\chi^2_1 = 2.16$, $p = 0.142$; interaction: LR $\chi^2_1 = 1.34$, $p = 0.247$) or 4 M family groups (GLM, group type: LR $\chi^2_1 = 18.54$, $p < 0.0001$; sex: LR $\chi^2_1 = 1.77$, $p = 0.183$; interaction: LR $\chi^2_1 = 0.91$, $p = 0.341$).

4. Discussion

In social insect systems where progeny are sired by different males, potential conflicts between patrilines emerge and may select against the expression of patriline-specific signatures in the CHC profiles of offspring. These conflicts are thought to constrain information content in the cues displayed by each group member and to limit the scope for nepotism between progeny of the same sire [18,19]. While previous research focused on eusocial systems (e.g. reviewed in [21]; but see [22–24]), we showed here that such constraints can also be found in an insect species with simpler forms of social life (maternal care and family life). In particular, our results demonstrated that mate number did not influence the inter-individual diversity of CHC profiles expressed among young earwig offspring, whereas it increased such diversity in the resulting groups of adult offspring. In other words, there was no significant information content on mate number among young nymphs, but this information was expressed among adult males and females. Our study also demonstrated that even if the CHC profiles of nymphs, adult males and adult females contained a heritable component that could mediate the recognition patterns formerly reported in this species in terms of cannibalism and food sharing [29,33], they also reflect to a lower extent the environment and social group experienced by the individuals.

Our results supported the prediction that family life and multiple mating should favour the concealment of paternally inherited cues only in the young offspring (i.e. during family life). We did not find evidence for a paternal signature in the form of increased variability in the CHC profiles of nymphs from multiply sired clutches but found such an increase among adult offspring. Because earwig males in our experiment never encountered the eggs or the offspring they sired [27], any paternal signature in offspring CHC profiles would reflect paternally inherited variation at least partly, irrespective of the proximate mechanisms underlying the expression of heritable variation in CHC profiles. Proximately, the inherited variation can be expressed due to, for example, genetic variation in the fat metabolism, the preference for certain micro-environments or food intake behaviour, which in turn may affect CHC profiles. Different potential expression pathways may affect the temporal stability of the expressed heritable variation, but it does not change the ultimate effect that heritable information about maternal and/or paternal origin is displayed.

One hypothesis to explain the observed lack of paternally inherited cues in the chemical profiles of the nymphs is that their expression is developmentally constrained at this stage. For instance, nymphs might only be able to express immature profiles, because the paternal-cue-coding part of their genotype can only be fully expressed after a certain maturation time. In line with this hypothesis, it was shown in several dipterous insects (*Cucilidae*, *Muscidae* and *Drosophilidae*) that the CHC profile does not remain constant throughout their life [3]. In the ant *Cataglyphis niger*, the amounts of hydrocarbons in the postpharyngeal gland increased with maturation, especially in the first 7 days after emergence [34]. An alternative hypothesis is that mothers conceal information about their offspring's paternal origin by transferring CHCs to the eggs during oogenesis, as reported in the German cockroach *Blatella germanica* [35], or continuously to the nymphs during the period of maternal care. In the European earwig, the continuous transfer of CHCs to the eggs [36] and the frequent maternal grooming of nymphs [37] could allow females to progressively shape nymph CHCs by applying hydrocarbons. Ultimately, maternal concealment of paternal signatures in the nymph CHC profiles may either reflect a side effect of maternal behaviour (e.g. body contact, grooming and food provisioning), or an evolved maternal strategy to limit nepotistic/antagonistic sibling interactions among the different patrilines inside her brood. Further research on the mechanism and adaptive function of the found patterns is needed. But consistent with the hypothesis that selection on mothers favoured concealment of paternally inherited signatures on her offspring, previous experiments showed discrimination in cannibalism among nymphs from different clutches (i.e. with different mothers) [29], but lack of effect of multiple mating on cannibalism rate within clutches [38].

Our results further demonstrated that the CHC profiles of *F. auricularia* adults not only have a heritable component, but also partly reflected the shared environment and social group. In particular, experimental groups of unrelated adults were successfully assigned to their new group (although at a significantly lower success rate compared with the family

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groups). It was shown before that multiple abiotic factors can influence the chemical profiles of individuals, such as temperature [39], nesting substrate [8,40] or diet [10]. As we kept abiotic factors constant between our groups, we consider them an unlikely influence on group-specific profiles. Hence, the most likely explanation for the reported result is that social interactions passively (e.g. body contacts) and/or actively (e.g. allogrooming) mediated the transfer of chemical compounds between adults and thus contributed to the partial homogenization of odours within groups, a common process in colonies of eusocial insects [41,42]. In *F. auricularia*, old nymphs and adults are known to aggregate densely for foraging, resting and mating [43,44], as well as to express allogrooming [45], which both offer scope for social transfer of chemical compounds. Social transfer of recognition cues within groups may be beneficial for instance because it can facilitate the expression of group-directed forms of social behaviour [46].

A somewhat surprising result from our analyses was that the family-specificity of CHC profiles was higher among male than female adult family groups, suggesting a sex-difference in the expressed heritable variation in CHCs or an enhanced CHC exchange between males as compared with females, e.g. owing to higher levels of allogrooming and close physical contacts [12,41,47]. Because we found no difference in the group-specificity of CHC of unrelated males versus unrelated females (figure 2), the sex difference in the expressed CHC variation is more likely owing to intrinsic differences between the sexes, that is, a difference in the expression of heritable variation as was found in *Drosophila simulans* [48].

The discovered patterns of expressed CHC variation in *F. auricularia* are consistent with a scenario where mothers conceal any paternal signature in their offspring's chemical profiles to minimize antagonistic interactions among patrilines inside her brood, and where later in life information about both maternal and paternal origin are expressed potentially to avoid costs of sibling-mating in adults. However, the mechanisms of how paternal signatures in kin recognition cues of juveniles are concealed, and their adaptive function, require further investigation. Overall, our results provide insight into the role of parental care and social life in the evolution of chemical communication and recognition cues.

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Data accessibility. Data are deposited in the Dryad repository (doi:10.5061/dryad.73180).

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Parent–offspring conflict and the genetic trade-offs shaping parental investment

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The genetic conflict between parents and their offspring is a cornerstone of kin selection theory and the gene-centred view of evolution, but whether it actually occurs in natural systems remains an open question. Conflict operates only if parenting is driven by genetic trade-offs between offspring performance and the parent's ability to raise additional offspring, and its expression critically depends on the shape of these trade-offs. Here we investigate the occurrence and nature of genetic conflict in an insect with maternal care, the earwig *Forficula auricularia*. Specifically, we test for a direct response to experimental selection on female future reproduction and correlated responses in current offspring survival, developmental rate and growth. The results demonstrate genetic trade-offs that differ in shape before and after hatching. Our study not only provides direct evidence for parent–offspring conflict but also highlights that conflict is not inevitable and critically depends on the genetic trade-offs shaping parental investment.

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Parenting takes time, resources and energy, and ultimately reduces the parent's ability to produce additional offspring. It only pays off evolutionarily because it enhances the fitness of offspring to which the parent is genetically related[1]. But parenting is not necessarily harmonious altruism. Sexual reproduction is thought to introduce genetic conflict between family members. Each offspring should demand more investment than parents are selected to provide because it is more related to itself than to any of its siblings, whereas parents are equally related to all of their offspring[2]. Although the premise of parent–offspring conflict was conceptually quickly confirmed and accepted after Trivers' original formulation in 1974[3–5], almost two decades later the lack of empirical tests was striking and the topic considered a 'case of arrested development'[6]. Godfray[7] identified the lack of testable predictions of the theory as the main problem and proposed a major shift in the research programme away from the conflict as such (that is, the 'conflict battleground'[7]) to how parents and offspring should behave to resolve conflict[7–9]. This approach triggered a great amount of experimental research on behavioural parent–offspring interactions that provided evidence broadly consistent with conflict (reviewed in refs 5,10–14). However, the downside of this approach was that it sidestepped the fundamental question whether genetic parent–offspring conflict actually occurs and, thus, whether its assumed prominent role as driver of parenting and family life is justified.

There are three main predictions that empirical tests of a Triversian parent–offspring conflict battleground have to address. First, the conflict is over parental investment (PI) and not over parenting behaviour. Thus, it is essential to quantify PI according to its ultimate definition, that is, to measure any investment by a parent that enhances offspring fitness at the expense of the parent's expectation for additional offspring (Fig. 1)[1,2,4,15]. Second, the conflict is among genes, not traits or behaviours, and therefore only occurs if PI is shaped by genetic rather than phenotypic trade-offs between parents and offspring. Hence, empirical tests should demonstrate that genotypes with enhanced performance as offspring exhibit reduced ability to raise many offspring as parents (due to higher PI), and *vice versa* for genotypes with reduced performance as offspring[16,17]. Finally, while genetic trade-offs provide evidence for antagonistic parent–offspring co-evolution, they *per se* are not sufficient evidence for parent–offspring conflict over the amount of PI. This conflict occurs when PI fitness optima differ for parent and offspring[2,7,14], a condition requiring sexual reproduction and depending on the shape of the genetic trade-offs. It is only occasionally reached when offspring fitness gains show constant or accelerating returns, but always met under diminishing returns, that is, when offspring stand to gain less from an additional unit of investment when they are already in good than when they are in poor condition (Fig. 1)[2,4,5,18]. Hence, experimental tests should investigate the presence and shape of the genetic trade-offs, with evidence for conflict being most compelling under diminishing returns.

Theoretically, PI contains on the one hand the trade-off between investment in current offspring and the parent's expectation of future offspring (potentially leading to between-clutch conflict), and on the other hand the reallocation of investment among offspring within clutches (potentially leading to within-clutch conflict)[5,19–21]. In this study, we focused on the former and tested the three above predictions using a large scale and replicated selection experiment in an insect with extended maternal care, the earwig *Forficula auricularia*. The genetic trade-offs shaping PI were investigated by exerting selection on the mothers and quantifying the correlated responses in offspring. *F. auricularia* is an ideal system for this study: the species reproduces sexually (a prerequisite for parent–offspring conflict[2]), females care for eggs and hatched nymphs, and they produce up to two clutches in their lifetime[22–24]. From the viewpoint of earwig females, first-clutch offspring are current offspring, the relative size of the second clutch is an estimate of the female's expectation for future offspring, and the relationship between the size of the second clutch and the performance of first-clutch offspring quantifies the trade-offs shaping PI. Finally, multiple paternity is common in earwigs[25], leading to variation in genetic relatedness within and between first and second clutches that can further mediate scope for conflict.

We selected females with low expectation of future offspring (that is, **S**mall relative size of (or no) second clutch; **S**-lines), high expectation of future offspring (that is, **L**arge relative size of second clutch; **L**-lines) and intermediate expectation of future

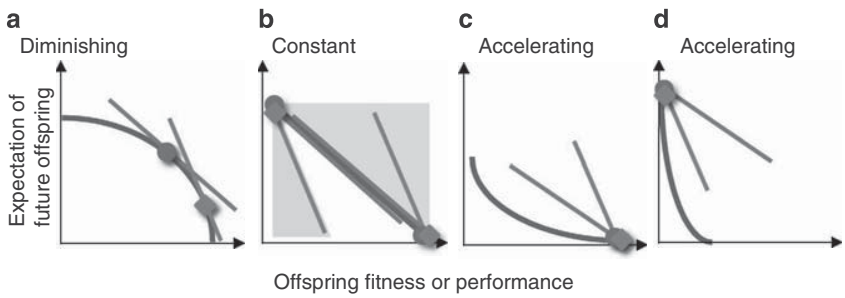

Figure 1 | Theoretical plots depicting how the shape of genetic trade-offs affect the parent–offspring conflict battleground. (**a**) Curved trade-off with diminishing returns (grey line). The intersection of the fitness isoclines (tangent lines) to this curve are optima and their slope is steeper for the offspring (red line) than for the parent (blue line) because each offspring is at least twice as related to itself than to its sibling, whereas the parent is equally related to all its offspring (slope for parent $= -1$; slope for offspring $= -2$ in case of full siblings[4]). The parent and offspring optima (blue circle and red diamond, respectively) differ and, thus, there is parent–offspring conflict over the amount of PI in current offspring (modified from ref. 4). (**b**) Linear trade-off with constant returns. When the trade-off lines have slopes that lay in the blue area, parent and offspring agree that the parent should not produce future offspring. Conversely when the trade-off lines have slopes that lay in the red area, parent and offspring agree that the parent should terminate PI and produce additional offspring. When the trade-off lines have slopes equivalent to the fitness isoclines, no optima occur and all combinations of parent and offspring values are equivalent. Only in the white area there is conflict; not over the quantitative partitioning of PI among offspring, but over whether or not future offspring should be produced. (**c**,**d**) Curved trade-off with accelerating returns. (**c**) When current offspring stand to gain substantially from PI, the parent should invest all its resources in current offspring, produce no future clutch and there is no conflict. (**d**) When current offspring do not gain much from further PI, the parent should terminate its investment, produce a second clutch and there is no conflict. Conflict can only occur for trade-off curves intermediate to (**c**) and (**d**); not over the quantitative partitioning of PI among offspring, but over whether or not future offspring should be produced.

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offspring (that is, **C**ontrol; **C**-lines) in ten independent experimental populations over the course of six generations. The experiment included a total of 2,720 females with their offspring (287,636 eggs and 214,815 nymphs of first and second clutches). We predicted a correlated response to selection in offspring performance that was antagonistic to the direct response in females, with increased performance in S-lines and decreased performance in L-lines. Offspring performance was followed by covering the periods of maternal care before and after hatching and including measures of developmental rate, growth and survival. Finally, we explored the shape of the genetic trade-offs emerging between selection lines in the last generation. Overall, our results demonstrate (1) the occurrence of genetic trade-offs between the mother's expectation of future offspring and several offspring performance traits expressed before and after hatching; and (2) diminishing returns for offspring performance before hatching, but constant returns after hatching when mothers and offspring interact. Our study provides clear evidence for a parent–offspring conflict battleground during the egg stage, and highlights that its occurrence and nature critically depends on the genetic trade-offs shaping PI.

Results

Direct response to selection in mothers. S-line females evolved towards a lower relative second-clutch size as compared with L-line females (Fig. 2a), as expected. Per generation, the S- and L-lines diverged by 0.106 s.d. units (Fig. 2b) resulting in a mean difference of 0.637 s.d. in generation six (Fig. 2a). This response was due to significant changes in the size of the second clutch, while the size of the first clutch did not change significantly (Table 1). Furthermore, S-line females gained significantly less mass within 14 days after hatching of their first clutch (Table 1), a morphological proxy predicting second-clutch production[24]. These findings together confirm that S-line females evolved lower expectation for future offspring production than L-line females.

Correlated responses to selection in offspring. Four performance traits of first-clutch offspring showed the antagonistic correlated responses to selection expected under a genetic trade-off. During the egg stage, hatching success and the rate of embryonic development increased in the S-line compared with

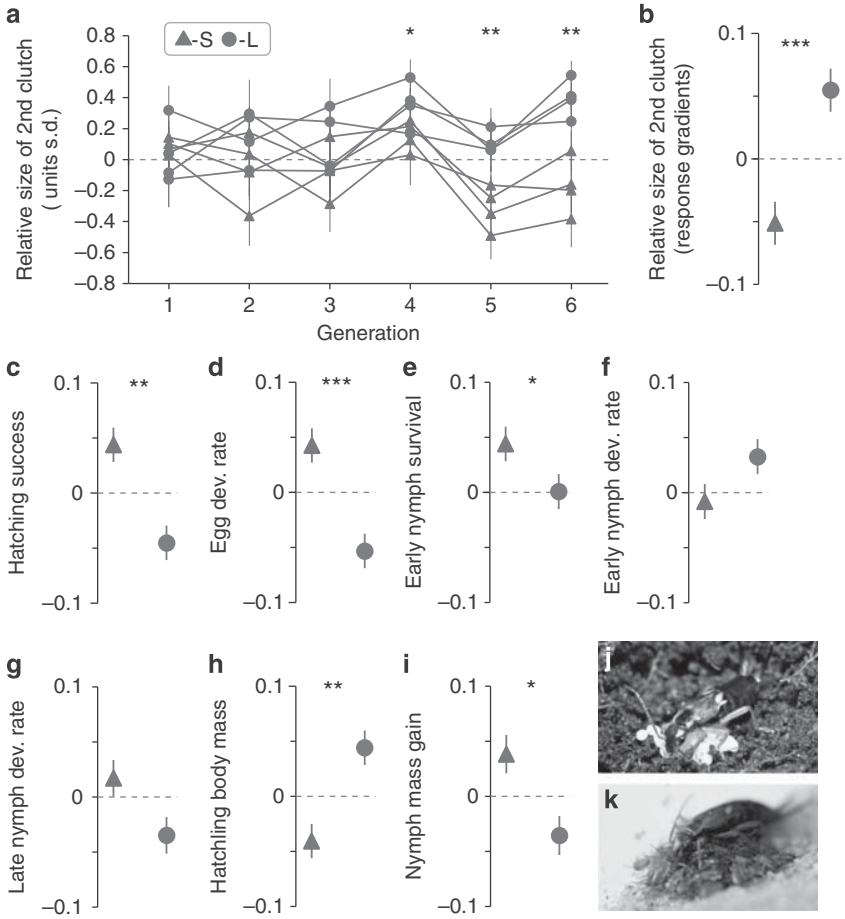

Figure 2 | Direct and correlated responses to selection. $N=4$ S-lines (red symbols and lines), $N=2$ C-lines and $N=4$ L-lines (blue symbols and lines) throughout. Direct response to selection as (**a**) time course of the mean (±s.e.m.) trait values per replicate selection line (population pair), computed as deviation from the mean of the two control (C) lines and (**b**) as linear response gradients (estimated using linear mixed models (LMMs); see 'Statistical analysis' in Methods section and Table 1; $n=2{,}289$ females with offspring). The correlated responses to selection in first-clutch offspring are displayed as linear response gradients: (**c**) proportion of hatched eggs ($n=2{,}628$); (**d**) egg developmental (dev.) rate between oviposition and hatching ($n=2{,}519$); (**e**) proportion of nymphs surviving from hatching until day 14 ($n=2{,}474$); (**f**) early nymph developmental rate from hatching to molt to second instar ($n=2{,}438$); (**g**) late nymph developmental rate from second instar to adult emergence ($n=2{,}228$); (**h**) mean nymph body mass 1 day after hatching ($n=2{,}507$); and (**i**) proportional nymph mass gain from hatching until day 14 ($n=1{,}415$). The scales on the y-axes are in units of s.d. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; LMM. (**j**) Picture of an earwig female tending her eggs, and (**k**) of a female tending her nymphs. Picture credits: J.M.

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Table 1 | Direct response to selection in earwig mothers and correlated responses to selection in their offspring.

	Selection treatment				Generation				Interaction				Pop-pair	Response gradients (±s.e.)	
	df1	*df2*	*F*	*P*	*df1*	*df2*	*F*	*P*	*df1*	*df2*	*F*	*P*	Varcomp (±s.e.)	S-lines	L-lines
Female															
Relative size of second clutch	2	7.04	10.23	**0.008**	1	2,278	0.02	0.887	2	2,279	7.54	**0.001**	0.0025 (0.0036)	−0.051 (0.017)	0.055 (0.017)
First-clutch size	2	7.03	2.35	0.165	1	2,707	0.16	0.692	2	2,707	0.58	0.560	0.0190 (0.0121)	−0.003 (0.015)	−0.015 (0.015)
Second-clutch size	2	7.06	2.08	0.195	1	2,277	0.02	0.883	2	2,277	3.82	**0.022**	0.0049 (0.0037)	−0.028 (0.012)	0.023 (0.012)
Likelihood second clutch	2	7.08	0.11	0.900	1	2,706	0.03	0.867	2	2,707	0.42	0.657	0.0033 (0.0037)	−0.014 (0.016)	0.005 (0.016)
Lifetime egg number	2	7.07	0.40	0.684	1	2,277	0.15	0.697	2	2,277	0.64	0.530	0.0148 (0.0092)	−0.013 (0.013)	0.008 (0.013)
Egg cannibalism*	2	7.03	0.16	0.858	1	2,707	0.15	0.694	2	2,707	3.92	**0.020**	0.0058 (0.0051)	−0.019 (0.016)	0.043 (0.016)
Body mass (d1)	2	7.06	1.08	0.389	1	2,504	0.16	0.684	2	2,504	2.64	0.072	0.0080 (0.0063)	0.036 (0.016)	−0.015 (0.016)
Mass gain (d1–d14)	2	7.20	2.39	0.160	1	2,454	0.30	0.584	2	2,454	6.68	**0.001**	0.0035 (0.0040)	−0.056 (0.016)	0.029 (0.016)
Offspring															
Hatching success	2	7.07	0.25	0.783	1	2,616	0.00	0.976	2	2,617	6.24	**0.002**	0.0023 (0.0033)	0.044 (0.016)	−0.045 (0.016)
Egg developmental rate	2	7.05	1.44	0.300	1	2,507	0.03	0.861	2	2,507	7.40	**0.001**	0.0062 (0.0054)	0.042 (0.016)	−0.054 (0.016)
Early nymph survival	2	7.02	0.88	0.458	1	2,462	0.74	0.390	2	2,462	4.50	**0.011**	0.0017 (0.0031)	0.046 (0.016)	0.002 (0.016)
Early nymph developmental rate	2	7.04	4.02	0.068	1	2,426	0.20	0.655	2	2,426	2.07	0.127	0.0034 (0.0040)	−0.008 (0.016)	0.033 (0.016)
Late nymph developmental rate	2	6.89	1.21	0.354	1	2,215	0.07	0.795	2	2,215	2.29	0.102	0.0163 (0.0110)	0.017 (0.017)	−0.035 (0.017)
Hatchling body mass	2	6.99	0.37	0.707	1	2,494	0.00	0.972	2	2,494	5.72	**0.003**	0.0274 (0.0164)	−0.041 (0.016)	0.044 (0.016)
Nymph mass gain†	2	7.21	2.83	0.124	1	1,405	0.02	0.897	2	1,405	3.37	**0.035**	0.0035 (0.0055)	0.038 (0.018)	−0.036 (0.018)

Results from linear mixed models (LMMs) on the standardized variables with the selection treatment as fixed factor, generation as linear covariate and the population pair as random effect. Data from six generations, $N=10$ population pairs (that is, selection lines) and a total of $n=2{,}720$ females (that is, families). Denominator degrees of freedom (*df2*) of different models may vary due to missing values of corresponding measurements. Provided are significance tests for the fixed effects and variance component estimates (±s.e.) for the random effect. Standardized response gradients were obtained as the regression coefficients from the interaction term between selection treatment and generation. They represent the linear slopes for S- and L-lines relative to the C-lines in units of s.d.. Significant ($\alpha=0.05$) *P* values are in bold.
*Female cannibalism of eggs was calculated as the difference in the number of eggs between oviposition and hatching[34]. Egg number at hatching was the sum of hatched nymphs and unhatched eggs.
†Data only available for F1, F2, F3 and F6 generations.

the L-line (Fig. 2c,d; Table 1). The effect on hatching success was partly mediated by filial cannibalism, as L-line females showed an increasing tendency for egg cannibalism compared with C- or S-line females (Table 1). After hatching, early nymph survival and their relative mass gain until day 14 showed the expected correlated responses, increasing in the S-lines relative to L-lines (Fig. 2e,i). The correlated responses in early and late nymph developmental rate were not significant (Fig. 2f,g) and nymph body mass at hatching decreased, rather than increased, in S-lines (Fig. 2h).

Shape of the genetic trade-offs. The shape of the trade-off curves was inferred from the relationships between the population means for the size of second clutches and the offspring performance traits across the three selection treatments (Fig. 3). Only the data from the last generation were used because the likelihood to detect diminishing returns, if present, is highest when mean trait values have diverged most. Qualitative evidence for a concave curved genetic trade-off and, thus, for diminishing returns and conflict was found for the egg stage in relation to hatching success and embryonic developmental rate (Fig. 3a,b). In contrast, the trade-offs after hatching with mass gain and nymph survival were approximately linear and indicated constant rather than diminishing returns (Fig. 3c,d). The slope with nymph mass gain was less steep than -1 (slope $=-0.63$), but steeper than -1 (while also less clearly linear) with regard to nymph survival (slope $=-1.37$).

Discussion

Behaviours in families are generally thought to be the outcome of a genetic conflict over parental investment. This conflict is a cornerstone of kin selection theory and the gene-centred view of evolution[2,7,26]. However, an empirical demonstration of the conflict battleground[7] has remained an unsolved difficulty to this day, partly due to intrinsic limitation of behavioural or phenotypic studies to demonstrate genetic conflict[6,12,14], and partly due to experimental difficulties of quantifying PI[27] and demonstrating different fitness optima for parents and offspring[7,14].

In this study, we addressed these open questions using a selection experiment in the earwig *F. auricularia* and show empirical evidence for genetic conflict between parent and offspring over PI, at least during the egg stage. More specifically, we show that experimentally selecting on the females' expectation for future offspring (that is, the relative size of their second clutch) resulted in a direct response in terms of second-clutch size and correlated antagonistic responses to selection in offspring performance traits. These results demonstrate genetic trade-offs shaping PI, which is an essential (albeit not sufficient; see introduction) precondition for conflict to occur. The direct and correlated responses to selection were consistent among replicate lines with small and nonsignificant variation between population pairs due to drift. Furthermore, different fitness optima for earwig mothers and offspring were inferred by examining the shape of the genetic trade-offs in the last generation. They showed diminishing returns during the egg stage revealing scope for parent–offspring conflict over hatching success and egg developmental rate. After hatching, the trade-offs were linear implying constant returns and a probably minor role for conflict over nymph survival and growth (see below).

The correlated responses to selection in offspring were in the direction predicted by genetic trade-offs with regard to four offspring performance traits. As compared with L-line offspring, S-line offspring evolved towards enhanced hatching success, faster egg development, higher nymph survival and mass gain. The trade-off with hatching success was partly due to L-line females evolving a higher tendency to cannibalize their eggs, which fits the expectation that females with higher expectation for future reproduction should prioritize somatic maintenance (that is, food intake by egg recycling) over parenting and current offspring survival[1,15]. The responses in egg developmental rate may be due to changes in maternally transferred hormones or resources in the eggs, which are common maternal effect mechanisms across taxa[28–30], or in maternal egg care behaviour[31]. The correlated responses of nymph survival and growth indicate enhanced post-hatching maternal care in S-line females, for example, through food provisioning[23,32] and/or maternal modulation of siblicide among nymphs. In earwigs, nymph mortality is partly due to siblicide[33] and, thus, the enhanced survival of nymphs in the S-line could also indicate a reduced siblicidal tendency of S-line nymphs. Compared with these four traits, the correlated response to selection in nymph body mass at hatching is less straightforward to interpret leaving

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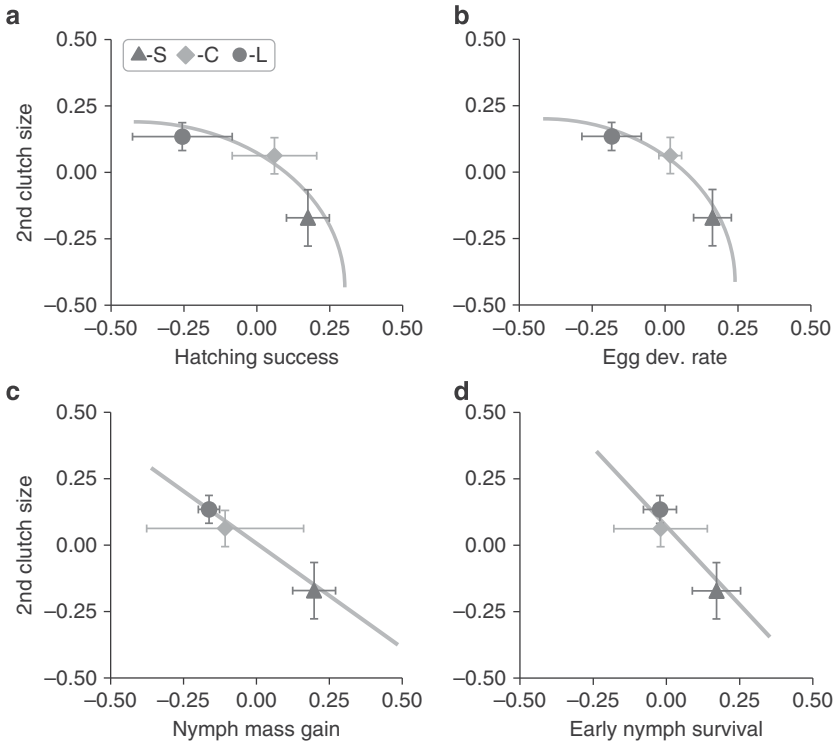

Figure 3 | Shape of genetic trade-offs between second (2nd) clutch size and offspring performance. Shown are the trait means (±s.e.m.) from the last generation (generation six) across the S-lines (red symbols; $N=4$ lines, $n=134$ families), the C-lines (yellow symbols; $N=2$ lines, $n=73$ families) and the L-lines (blue symbols; $N=4$ lines, $n=145$ families). Curved trade-offs with diminishing returns before hatching for (**a**) hatching success and (**b**) egg developmental (dev.) rate. Linear trade-offs with constant returns after hatching for (**c**) nymph mass gain (slope (±s.e.) = −0.63 (0.01)) and (**d**) nymph survival (slope (±s.e.) = −1.37 (0.31)).

room for two alternative interpretations. It could either reflect more maternal care during the egg stage by S-line females because attended eggs are known to develop into lighter hatchlings than orphaned eggs[31], possibly due to the selective survival of heavier hatchlings under low levels of egg care. In this case, the observed response would be according to the predictions of a trade-off. Alternatively, because hatchlings from smaller eggs tend to be lighter[34], S-line females may produce smaller eggs, which would be opposite to prediction. Given the straightforward interpretation of the first four offspring performance traits as components of the genetic trade-offs shaping PI, we focused on these in our examination for diminishing returns and scope for conflict.

The shape of the genetic trade-offs was inferred by comparing the evolutionarily diverged offspring performance traits and relative size of the females' second clutches between the three selection treatments. The curved genetic trade-offs during the egg stage indicate diminishing returns providing evidence for conflict over hatching success and egg developmental rate. Specifically, the increase in hatching success/developmental rate per unit decrease in the size of the female's second clutch was less between the C- and S-lines (high offspring performance) than between the C- and L-lines (low offspring performance). At first view, conflict during the egg stage may be thought to have little evolutionary consequence because the eggs are developmentally constrained in their ability to influence PI, and part of the conflict was due to female filial cannibalism that eggs cannot prevent. However, embryos are known to respond developmentally to other, more subtle forms of maternal influences (for example, maternal hormones in the eggs), and conflict can operate on these mechanisms[29,30]. The potential occurrence, scope and function of such maternal effect mechanisms remain to be investigated in *F. auricularia*.

Despite genetic trade-offs, the evidence for conflict was weak after hatching when earwig mothers provide food to their young and nymphs signal their condition by solicitation pheromones[32]. The trade-off curves with nymph mass gain and survival were approximately linear indicating constant returns. Under constant returns, scope for conflict is limited and, if it is predicted, it is not over the partitioning of the amount of PI, but over whether or not the mother produces a second clutch (Fig. 1). The slope of the trade-off line was less steep than −1 for mass gain, which implies that with regard to effects on this offspring trait, earwig mothers and nymphs agree that females should not produce a second clutch (which could explain why a fraction of earwig females produces only one clutch in their lifetime[24]). For nymph survival the slope was steeper possibly in the range of mother–offspring conflict over second-clutch production. Indeed, our former research demonstrated that nymphs can influence whether or not caring females produce a second clutch, mediated by a paternally inherited effect[35]. Thus, whether or not earwig females produce a second clutch may have partly evolved due to the genetic trade-offs with nymph growth and survival. Our result that scope for conflict is more limited after than before hatching is somewhat surprising because parental feeding and offspring begging is the classical context used to model how parents and offspring should behave to resolve conflict[5,7,8,12,13], where diminishing returns are commonly assumed, and thus the one where conflict over the amount of PI is *a priori* most expected.

By selecting on the relative size of second clutches, we focused on genetic trade-offs operating between clutches, which can drive conflict over PI among successive breeding attempts as originally

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envisaged by Trivers[2]. S-line nymphs evolved towards a higher mean offspring performance, without a significant change in the size of first clutches, which confirms the prominent role of the between-clutch trade-off for PI and scope for this form of conflict in *F. auricularia*.

Our findings highlight that the nature of conflict depends on genetic trade-offs and that conflict is not inevitable. Parent and offspring behaviours may also be driven by antagonistic mother–offspring co-evolution with no or minor influences of conflict. Such a process should result in co-adapted[17,36] and well-coordinated parenting with low-cost honest begging[6]. Thereby, the genetic link between parental reproduction and offspring performance allows PI to quickly evolve and adapt in a changing environment.

From a life-history perspective, the constant returns and weak evidence for conflict after hatching, as compared with the egg stage, may at least partly reflect the partial independence of earwig nymphs from their mother's care during this stage[23]. Partial independence may limit conflict as compared with systems where offspring are fully dependent on their parents, such as altricial birds or mammals. Under partial independence, constant returns may be more likely because low levels of care have less devastating effects on offspring performance than under full offspring dependence and obligate care. If correct, this hypothesis would imply that parent–offspring conflict had limited impact in the early evolution of parenting when offspring did not fully depend on their parents and that, if present, conflict was mainly over whether or not parents should reproduce again (that is, their parity). More generally, the biological importance of genetic conflict should depend on factors determining the curvature of the genetic trade-offs shaping PI such as the life history and possibly also ecology of a population/species.

In conclusion, our study shows clear evidence for a genetic conflict between parents and offspring over PI. It thereby solves a long-standing problem that was previously conceived prohibitively difficult to address and, thus, fills a major gap in our empirical proof of concepts in the evolution of behaviours in families. Furthermore, and contrary to former thought, our results also reveal that conflict may not globally and *a priori* be assumed to be the major driver of parenting and family life. The nature and scope for conflict critically depends on the shape of the genetic trade-offs underlying PI, which needs empirical testing, and PI may also evolve by conflict-free antagonistic parent–offspring co-evolution enabling PI to evolve as coadapted and well-coordinated parenting and family life.

Methods

Laboratory breeding. The animals forming the base population of this selection experiment were caught from a wild population in early June 2009 in Dolcedo, Liguria/Italy (7° 56′ 55″ E, 43° 54′ 14″ N, altitude 78 m a.s.l.). It consisted of ~1,200 predominately fourth juvenile instars and recently emerged adults. After transfer to the laboratory, the field-caught individuals were assigned randomly to 20 mating groups of 60 individuals each (30 females and 30 males) and kept separately in plastic containers for mating (see ref. 24 for a detailed description of the base population). The artificial selection experiment was initiated based on the progeny of these field-caught animals, that is, the first laboratory-born generation of adults (F1). Upon emergence as adults, the F1 males and females were randomly assigned to 20 mating groups of 48 individuals each (24 females and 24 males). The mating groups were held in plastic containers (dimensions: 37 × 22 × 25 cm) with humid sand as substrate and with egg cardboard and plastic tubes as shelters. The containers were lined with fluon and covered with nylon thighs to prevent escape of the animals. They were fed with our standard laboratory food (a food jelly made from 20 g egg yolk, 60 g wheat germ, 120 g carrots, 60 g bird food, 60 g dry cat food, 60 g flower pollen, 40 g Agar, 1,800 ml water, 2 g ascorbic acid and 2 g sorbic acid) with adequately sized pieces twice a week (see also ref. 24).

The mating groups were held in climate chambers at a light:dark photoperiod schedule of 14:10 h and at a constant temperature of 20 °C (to which we refer as 'summer conditions') with relative humidity kept between 60 and 80%. As soon as at least two females from two different mating groups laid eggs, all females from all mating groups were set-up individually in Petri dishes (10 × 2 cm). The dishes contained humid sand as a substrate and a plastic tube as shelter. All females were kept for 7 days at 10 °C (no light) and then at 15 °C (no light) for oviposition and for the duration of egg care until hatching. Such 'winter conditions' are required to terminate the diapause of the eggs and trigger embryonic development[23,24]. Each female was provided food twice a week until oviposition, and no food was provided during egg care until hatching[23]. On day 1 after hatching, we set-up the hatched nymphs with their mother in a new Petri dish (10 × 2 cm) and returned them to 'summer conditions' (see above). During the first 2 weeks after hatching (that is, from day 1 until day 14), food was provided every other day. On day 14, females were separated from their nymphs and set-up in a new Petri dish (10 × 2 cm) for production of the second clutch (if any). Also on day 14, a total of 20 of her nymphs (or fewer in case of smaller nymph numbers) were chosen haphazardly

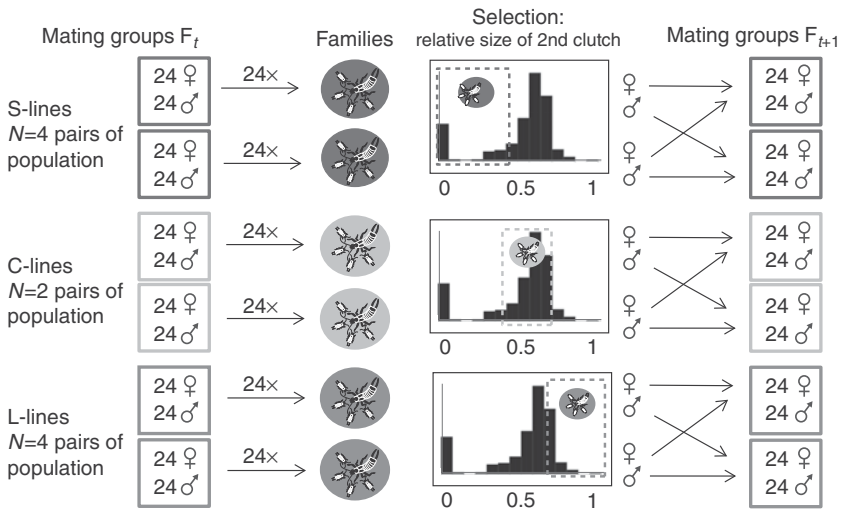

Figure 4 | Illustration of breeding design. Each box to the left represents a mating group in generation F_t, and to the right the mating group in the next generation F_{t+1}. Two mating groups together (randomly assigned at begin of experiment) formed one population pair (that is, selection line) each, out of a total of ten population pairs. Red boxes, S-line; yellow boxes, C-line; blue boxes, L-lines. In generation *t* females, the distribution of the relative size of their second clutches was assessed for each mating group (histograms). Sons and daughters were then selected according to their mother's value for the selection target (selection depicted by dashed frames). The new mating groups in generation $t+1$ were formed of the daughters from the females from that same mating group in the previous generation, and the sons of the females from the other mating group of this population pair to prevent brother–sister mating. The experiment was run over six generations.

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and set-up in larger Petri dishes (14.5 × 2 cm) where they were reared as family groups until adulthood. After day 14, both females and nymphs were fed twice a week.

If a female produced no second clutch within 60 days after hatching of the first clutch, the female was considered to produce only one clutch in her lifetime[24]. If the female produced a second clutch, we took the performance measures of second-clutch offspring up until hatching (see section 'Trait measurements' below). We did not rear any second-clutch offspring into adulthood. These basic procedures were applied to all generations of the selection experiment. The selection experiment was carried out over the course of six generations between spring 2010 and fall 2013.

Experimental design. A graphical illustration of the experimental design can be found in Fig. 4. The selection experiment was initiated after one generation of laboratory breeding without selection to reduce a potential impact of environmental variation modifying the response to selection through maternal effects[37]. Of each brood produced by the 24 F1 females of each of the 20 mating groups, a female and a male were randomly selected to form the new 20 mating groups. The number of individuals per mating group was 24 females and 24 males across all generations of the selection experiment. To avoid brother–sister mating and minimize potential effects of inbreeding depression due to sib-mating, the 20 mating groups were randomly assigned into paired populations among which the females and males were exchanged each generation to form the mating groups of the next generation. For example, the female progeny of former population A were set-up with the male progeny of former population B to form the new population A (and *vice versa* for the new population B). The assignment of mating groups into population pairs was established at set-up of the field-caught individuals (F_0) and was maintained over the whole course of the selection experiment. In this selection design, the unit of replication (that is, the selection line) is the paired population as it defines the independent gene pools that may evolve in response to selection.

From the total of 10 population pairs (that is, replicate selection lines), four were selected for a relatively small second clutch ('S-lines'), four for a relatively large second clutch ('L-lines') and two for an intermediate relative size of the second clutch (control 'C-lines'). The relative size of the second clutch was computed as the number of eggs in the second clutch divided by the sum of eggs in the first and second clutches (the sum corresponding to the lifetime number of eggs in *F. auricularia*[24]). In the S-lines, we selected the bottom 50% (including females producing a single clutch), in the L-lines the top 50% and in the C-lines the intermediate 50% of the distribution in the relative size of second clutches among females of each mating group.

Although the relative size of the second clutch is a maternal trait with sex-limited expression, we applied selection through both sexes by using sons and daughters of the selected females/families (Fig. 4). We aimed at selecting two sons and two daughters of each selected female/family to keep mating groups of constant size (that is, 24 females and 24 males). This was not always possible due to cases of juvenile mortality, hatching failure or insufficient individuals from both sexes upon adult emergence in some of the families. In these cases, the number of selected individuals per brood/sex was adjusted by balancing stronger selection (using more individuals from mothers with the best fit to the selection criterion) against maintenance of genetic variability (using individuals from as many families as possible). The mean (± s.d.) numbers of females and males per family used over the six generations were 2.46 (0.86) and 2.50 (0.91), respectively. Only progeny from first clutches were used for breeding.

Trait measurements. We took various measures of offspring performance including estimates of survival (separate for eggs/embryos and nymphs), estimates of developmental rate (separate for eggs/embryos, early nymphs (hatching—second instar) and late nymphs (second instar—adulthood)) and estimates of growth (separate for body mass at hatching and body mass gain during the first 14 days after hatching, as measure of growth after hatching). Survival is a direct component of fitness, and mass gain and fast development gives nymphs a headstart in competitive/cannibalistic interactions[38,39]. In addition, a range of reproductive parameters was recorded. The oviposition and hatching dates for first and second clutches were taken upon observation of the first eggs of a female and corresponded to the date of first observation of egg laying or hatching in a given clutch, respectively. Clutch sizes were determined by counting the number of eggs of the first and second clutches for each female 1 day after the first observation of the start of oviposition. Similarly, the number of hatched nymphs was counted 1 day after observation of the first hatched nymph in a clutch. Because hatching is sometimes asynchronous, the unhatched eggs were kept for another day to count further hatched nymphs (if any) on the subsequent day, and the number of unhatched eggs was also counted. The total number of hatched nymphs over the 2 days as proportion of clutch size was used to quantify hatching success.

Earwig females sometimes cannibalize some of their eggs during the period of egg care[34]. To obtain a quantity of egg cannibalism, the sum of the hatched nymphs and remaining unhatched eggs at hatching was compared with the original clutch size. Any reduction in the number of eggs between oviposition and hatching is most likely due to maternal egg cannibalism, and the difference in progeny number between oviposition and hatching was used as a measure of filial egg cannibalism in the analysis.

The body mass of nymphs was measured twice, 1 day after hatching and on day 14 after hatching. For each clutch, ten haphazardly chosen nymphs were jointly added to an Eppendorf tube and the tube was weighed with and without the nymphs. The difference divided by ten was taken as the average nymph body mass of a given clutch. Hatchling body mass was taken in all generations. Body mass at day 14 was only available for generations F1, F2, F3 and F6. The relative mass gain of nymphs over the course of the first 2 weeks after hatching was calculated as the proportional increase in mass relative to the body mass at hatching. We also took two measurement of female body mass, once at hatching and once 14 days after hatching. The weight gain of females from hatching of the first clutch until day 14 is a predictor for the likelihood and size of the second clutch[24]. All mass measurement were done to the nearest 0.01 mg using a Mettler-Toledo MT5 Micro-balance (Mettler, Roche, Basel). For measures of developmental rate we calculated the number of days between egg laying and hatching (egg developmental rate), the number of days between hatching and the first nymph in a clutch molting into second instar (early nymph development), and the number of days between second instar to the first adult emergence in a clutch (late nymph development).

Statistical analysis. All variables were standardized to a mean of zero and unit variance within each generation for homogeneous variances across generations. To test for divergence of maternal and offspring traits between selection lines, we estimated standardized linear response gradients over the course of the six generations using linear mixed models and restricted maximum likelihood estimation. The trait of interest (standardized) was entered as the dependent variable, the selection treatment as fixed factor (H-lines, C-lines and L-line), the generation as continuous variable (linear term), the interaction between the selection treatment and generation as fixed factor and the paired populations (for example, 'A–B') as random effect. A linear response to selection is in this model demonstrated by a significant interaction between the selection treatment and generation. The regression coefficients from this interaction term are standardized linear response gradients, that is, the slopes of the linear trend for the S- and L-lines relative to the control C-line. Standardized response gradients estimate the per-generation change in population mean trait values expressed in units of s.d.. The random effect (the paired population) accounted for the dependencies of individuals from the same selection line (that is, sharing the same gene pool) and for differences between lines within selection treatments arising for reasons other than selection as, for example, genetic drift. Proportional variables (relative size of second clutches, hatching success and nymph survival) were logit-transformed[40] before standardization and analysis, and measures of developmental rate were computed by multiplying the standardized values of duration (number of days) by minus one, such that large positive values corresponded to fast development and large negative values to slow development. All reported P values are two tailed with a significance threshold α of 0.05. The statistical analyses were carried out using JMP PRO V11.0 statistical software (SAS Institute, Inc.).

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Author contributions

M.K. conceived the study and analysed the data; M.K. and J.M. designed the experiment and wrote the manuscript; S.B., J.M., J.W.Y.W., L.R. and D.S. managed the selection lines; and all authors collected the data and contributed to manuscript revisions with comments.

Additional information

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Parental antagonism and parent–offspring co-adaptation interact to shape family life

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The family is an arena for conflicts between offspring, mothers and fathers that need resolving to promote the evolution of parental care and the maintenance of family life. Co-adaptation is known to contribute to the resolution of parent–offspring conflict over parental care by selecting for combinations of offspring demand and parental supply that match to maximize the fitness of family members. However, multiple paternity and differences in the level of care provided by mothers and fathers can generate antagonistic selection on offspring demand (mediated, for example, by genomic imprinting) and possibly hamper co-adaptation. While parent–offspring co-adaptation and parental antagonism are commonly considered two major processes in the evolution of family life, their co-occurrence and the evolutionary consequences of their joint action are poorly understood. Here, we demonstrate the simultaneous and entangled effects of these two processes on outcomes of family interactions, using a series of breeding experiments in the European earwig, *Forficula auricularia*, an insect species with uniparental female care. As predicted from parental antagonism, we show that paternally inherited effects expressed in offspring influence both maternal care and maternal investment in future reproduction. However, and as expected from the entangled effects of parental antagonism and co-adaptation, these effects critically depended on postnatal interactions with caring females and maternally inherited effects expressed in offspring. Our results demonstrate that parent–offspring co-adaptation and parental antagonism are entangled key drivers in the evolution of family life that cannot be fully understood in isolation.

Keywords: facultative parental care; parental investment; food provisioning; conflicts; insect; *Forficula auricularia*

1. INTRODUCTION

Parental care is an important source of conflicts between offspring and parents due to asymmetries in the benefits of care to offspring and the costs of care to parents (e.g. in terms of individual future reproduction [1–4]). Parent–offspring co-adaptation is an evolutionary mechanism known to contribute to the resolution of parent–offspring conflict over parental care by selecting for combinations of offspring demand and parental supply that match to maximize the fitness of family members [5,6]. Co-adaptation occurs because individuals adapt to the parental supply when they are offspring and to the demand they inherit to their own offspring when they are parent [4–7].

Whereas parent–offspring co-adaptation is considered an important process in the evolution of family interactions [4,8,9], it remains unclear to what extent co-adaptation can operate when the two parents exhibit asymmetries in their investment or relatedness towards current offspring (e.g. due to multiple paternity), both of which commonly characterize animal mating systems [10]. For instance, the exclusive interaction between mothers (the caring parent in many taxa) and offspring in uniparental families frees fathers from the pressure to adapt to offspring demand and consequently excludes fathers and paternally inherited effects expressed in offspring from the co-adaptation process [11]. Furthermore, when multiple males sire the progeny of a female, parental antagonism is predicted to select for parent-of-origin specific inheritance mechanisms (e.g. through genomic imprinting [9,12,13] as shown in humans and mice [14–18]), which potentially hamper mother–offspring co-adaptation by preventing females from adapting to an offspring demand mainly shaped by paternally inherited effects [5,6]. In such families, paternally inherited genes expressed in offspring are predicted to selfishly exaggerate offspring demand because siblings are not equally related from their paternal-side and the different patrilines compete among each other for access to the provided maternal care. Conversely, maternally inherited genes expressed in offspring are predicted to limit the level of offspring demand because females suffer from the costs of exaggerated offspring demand and have siblings that are equally related from their maternal side [9]. While a growing number of experimental and theoretical studies show that parental antagonism and parent–offspring co-adaptation are keystones in the evolution of family life [4,8,9], little is known about their simultaneous effects within families and its consequence on family interactions.

Unravelling the simultaneous effects of co-adaptation and parental antagonism on family life requires manipulating the potential sources of conflict by forming families with alternative combinations of maternal, paternal and offspring strategies. A powerful experimental method is to conduct cross-breeding and cross-fostering [7,19,20] using individuals from a population where both evolutionary processes can fully operate to generate

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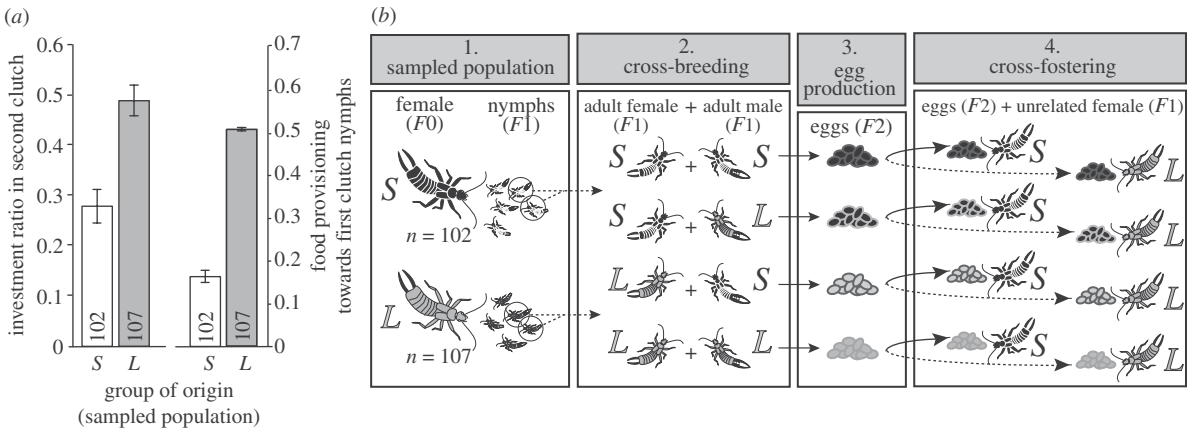

Figure 1. Natural variation in earwig family life and experimental design. (*a*) When compared with *S*-group individuals, *L*-group ones had mothers that exhibited larger investment in second clutch (egg number in second clutch divided by total egg number; Welch *t*-test, $t_{113.5} = 23.4$, $p < 0.0001$) and larger food provisioning (ratio of nymphs that get food from their mother 7 days after hatching; Welch *t*-test, $t_{125.7} = 4.70$, $p < 0.0001$). Error bars represent SEM. Sample size is at the bottom of each bar. (*b*) Details of the experimental design.

or maintain variation in family life. The European earwig, *Forficula auricularia*, is an ideal biological model to disentangle the effects of parental antagonism and mother–offspring co-adaptation on family life. In this species, females do not discriminate between their own and foreign nymphs, mate multiply, protect their eggs and nymphs against natural enemies and provide food to their young during approximately two weeks after hatching [21,22]. Furthermore, this species exhibits ample natural variation in family traits such as female investment in future reproduction (production of either one or two clutches during lifetime [22]) and level of food provisioning towards first clutch nymphs [22,23], which may be shaped by co-adaptation and parental antagonism.

In this study, we carried out a series of cross-breeding and cross-fostering experiments between two groups of earwigs that exhibit potential alternative outcomes of co-adaptation and/or parental antagonism [22]. The selected individuals were offspring from females sampled in a single natural population and exhibiting either small (*S*-group) or large (*L*-group) investment in the second clutch (figure 1*a*). These groups were also characterized by small and large levels of food provisioning towards first clutch nymphs, respectively (figure 1*a*). Scope for conflicts was manipulated by setting up eight types of experimental families wherein the origins of foster mothers (OFM) and of genetic mothers (OGM) and genetic fathers (OGF) of offspring form all possible matched and mismatched combinations of *S*- and *L*- origins (figure 1*b*). Maternal care and fitness correlates of mothers and offspring were then measured in these families.

Under the mother–offspring co-adaptation hypothesis, an interaction between OFM and OGM is predicted to significantly influence the measured traits, with lower values of maternal care and fitness correlates in mismatched than matched combinations [4,24]. Conversely, under the parental antagonism hypothesis, OGM, OGF and/or an interaction between these two factors (indicative of intragenomic epistasis [9,12]) are expected to significantly influence the measured values of maternal care and fitness correlates of mothers and offspring. For instance, if fathers control the level of maternal care through paternally inherited offspring effects (mediated e.g. through genomic imprinting), offspring sired by fathers from *L*-groups (OGF-*L*) are expected to receive more food than offspring sired by fathers from *S*-group (OGF-*S*). Finally, under the simultaneous and entangled effects of mother–offspring co-adaptation and parental antagonism, a two-way interaction between OFM and OGF (indicative of social epistasis [7,25]) or a three-way interaction between OFM, OGM and OGF are predicted to significantly influence our measurements. Overall, our results demonstrate that co-adaptation and parental antagonism are entangled key drivers of family interactions that cannot be fully understood in isolation.

2. MATERIAL AND METHODS

(a) *The study animals*

Males and females used for the breeding design were a first laboratory-born generation (*F*1) of *F. auricularia* (figure 1*b*). They were the offspring of 209 earwig *F*0 females (out of a total of 492 used in another experiment [22]) collected as fourth instars nymphs or newly emerged adults in early June 2009 in a natural population located in Dolcedo, Italy. The laboratory rearing of earwigs is detailed in [22]. Briefly, *F*0 females were set up in plastic containers and reared under standardized laboratory conditions, where they randomly mated with males sampled at the same location. Approximately three months after setup, these females were isolated in Petri dishes (10 × 2 cm) to induce clutch production. Fourteen days after hatching of the first clutch of each female, 20 nymphs per family were isolated in new Petri dishes (= first-clutch families) and reared until adulthood, while the mothers were setup in new Petri dishes to allow second-clutch production. Upon the emergence of *F*1 adults, brothers and sisters from each first clutch were separated to prevent sib-mating. After the 492 *F*0 mothers produced a second clutch or were characterized as single-clutch females (females are unlikely to produce additional clutches 60 days after the

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hatching of their first clutch [26]), *F*1 adults of the first-clutch families were assigned to two experimental groups, depending on their mother's relative investment in second clutches, as being in the bottom third (*S*-groups, including both single- and double-clutch producers) or in the top third (*L*-groups, including only double-clutch producers) of the distribution (each group includes 164 families, figure 1*a*). This relative investment, which is the number of eggs produced in second clutch divided by the total number of eggs produced, measures how mothers invested in second relative to first reproduction, while controlling for their overall capacity of egg production.

(b) *Cross-breeding and cross-fostering*

The experimental design is detailed in figure 1*b*. The reciprocal breeding took place approximately one month after the emergence of *F*1 adults and involved individuals originating from 102 *S*-groups and 107 *L*-groups (among which 127 contributed to the experiment with both one male and one female, and 82 with either one male or one female). It was conducted by placing each virgin *F*1 female with one unrelated virgin *F*1 male in Petri dishes containing humid sand, a plastic shelter used as a nest and ad libitum artificial diet (see food composition in [22]). Mating pairs were assigned according to a full-factorial design with males/females being either *L*/*L* ($n = 37$), *L*/*S* ($n = 44$), *S*/*L* ($n = 40$) or *S*/*S* ($n = 48$), see figure 1*b*. Each pair was allowed to freely mate for three months, while their Petri dishes were maintained in a climatic chamber with 14 : 10 h/20 : 15°C light:dark photoperiod cycle. Females were then isolated, placed in complete darkness at 10°C for one week, and then in complete darkness at 15°C conditions until egg-laying and hatching.

Approximately two days after *F*1 females laid their first clutch, we cross-fostered eggs among them to obtain all possible combinations between OGM, OGF and OFM, i.e. a total of eight combinations (figure 1*b*). Foster mothers were always unrelated to the tended eggs. Each of the eight combinations contained mean ± s.e. = 21.1 ± 0.9 replicates. To ensure a balanced experimental design, each combination involved an equal number of foster mothers previously mated with males from *S*- and *L*-groups. To limit handling stress on females, eggs were transferred while females remained in their original Petri dish. No food was provided from egg-laying to hatching [22]. From day 1 after hatching to day 14, Petri dishes received ad libitum artificial diet every other day and were kept under 14 : 10 h light : dark photoperiod and a constant temperature of 20°C. At day 14, mothers were individually setup in new Petri dishes, where they had the possibility to produce a second clutch within the next 46 days (resulting in the total maximal interval of 60 days after hatching of first clutches; see above).

(c) *Measurements*

A total of five measures were taken on nymphs and mothers using standard procedures [22]. The number of eggs and nymphs produced in the first clutch of each female was counted 1 day after egg-laying and hatching, respectively. Foster mothers that did not lay a second clutch within the 60 days following the hatching of their first clutch were defined as single-clutch producers [22]. The survival rate of first clutch nymphs was calculated as the number of nymphs alive at day 14 divided by the total number of nymphs at hatching. The developmental time for first clutch nymphs was estimated by counting the number of days from hatching to the first observation of a second instars nymph in a clutch, a good measure of mean developmental time of the entire brood [22]. Finally, food provisioning towards first clutch nymphs was estimated using four successive steps that started at day 5 and consisted in (i) food depriving mothers and nymphs for 24 h, (ii) isolating females for 1 h while offering them green-coloured food, (iii) putting each female back in contact with 20 of its foster nymphs for 15 h, and (iv) calculating the proportion of nymphs with green gut [22,27]. The actual number of nymphs tended by foster mothers was not significantly correlated with our measure of food provisioning to the 20 nymphs (Pearson correlation test, $r = -0.053$, $p = 0.51$). Food provisioning could not be quantified in two cases owing to the small number of nymphs in the clutch (two and three nymphs, respectively), and in six cases that had mistakenly not been food-deprived on day 5.

(d) *Statistical analyses*

The effects of OFM, OGM, OGF and their interactions were tested on the maternal food provisioning, the likelihood of second clutch production by foster mothers, the relative investment of females into second clutch (in females producing a second clutch), as well as on developmental time and early survival rate of offspring using the statistical models described in table 1. Because previous results reported that some of these traits can be sensitive to variation in clutch size [22], this factor was included as covariate in the model of likelihood of second clutch production, where it refers to the number of first-clutch eggs produced by foster mothers, and in the models of developmental time of nymphs and survival rate of nymphs, where it refers to the number of nymphs attended by foster mothers. Clutch size was not entered in the model of food provisioning because the number of nymphs was standardized for food provisioning measurements and does not correlate with initial clutch size, and in the model of relative investment in second clutch as it was part of the response variable. Because of the strong asymmetry between the number of females that produced and did not produce two clutches during our experiments, the model on second-clutch production (likelihood of second clutch production, table 1) was based on a cloglog-link function [28]. All models were tested for overdispersion and corrected using quasi-GLM models when necessary [28]. To allow for a direct comparison of each tested factor across the five analyses, only four- and three-way interactions that were non-significant across all the statistical models were removed (model simplification based on AIC criteria). Note that results do not qualitatively change when models were simplified individually. All statistical analyses were conducted using the software R v. 2.14.0 (http://www.r-project.org/).

3. RESULTS

(a) *Food provisioning*

As predicted under the entangled effects of co-adaptation and parental antagonism, we find that food provisioning depended on the combined influences of OFM, OGM and OGF, a result shown by the significant three-way interaction between these factors (table 1A). Two non-mutually exclusive evolutionary scenarios could underlie this result. First, mother–offspring co-adaptation may protect mothers from paternally inherited offspring

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Table 1. Effects of OFM, OGM and OGF on five family performance traits. Significant *p*-values are in bold.

	A		**B**		**C**		**D**		**E**	
	food provisioning		likelihood of second clutch production		relative investment in second clutch[a]		developmental time of nymphs		survival rate of nymphs	
	LR	*p*	LR	*p*	LR	*p*	*F*	*p*	LR	*p*
size of 1st clutch (CS)	—	—	**9.46**	**0.002**	—	—	**24.93**	**<0.0001**	3.35	0.067
origin of foster mothers (OFM)	2.32	0.128	0.11	0.735	0.82	0.365	0.12	0.727	0.64	0.426
origin of genetic mothers (OGM)	0.62	0.431	<0.01	0.950	**4.42**	**0.036**	0.06	0.800	0.01	0.908
origin of genetic fathers (OGF)	**4.16**	**0.041**	0.82	0.365	0.42	0.518	1.79	0.183	1.38	0.240
CS:OFM	—	—	2.60	0.107	—	—	<0.01	0.989	**9.67**	**0.002**
CS:OGM	—	—	1.07	0.301	—	—	1.04	0.310	0.10	0.756
CS:OGF	—	—	3.71	0.054	—	—	**7.54**	**0.007**	0.68	0.410
OFM:OGM	0.62	0.432	0.93	0.334	0.20	0.658	0.51	0.474	<0.01	0.986
OFM:OGF	0.08	0.775	**15.67**	**<0.0001**	0.42	0.517	0.48	0.490	<0.01	0.951
OGM:OGF	<0.01	0.956	<0.01	0.972	1.11	0.293	0.15	0.700	0.02	0.895
CS:OFM:OGF	—	—	0.36	0.549	—	—	1.62	0.205	1.93	0.164
OFM:OGM:OGF	**5.04**	**0.025**	0.67	0.412	0.45	0.501	1.49	0.224	0.46	0.496
type of statistical model d.f. or *n*	Binomial GLM $n = 161$		Binomial GLM $n = 169$		Binomial GLM $n = 144$		GLM d.f. = 1,156		Binomial GLM $n = 169$	

[a]In females that produced two clutches during the experiment.

effects on provisioning. In line with this hypothesis, OGF had no significant effect on food provisioning in families where OFM and OGM matched (figure 2*a*; binomial GLM, $n = 80$; OGF: likelihood ratio, LR, $\chi^2 = 0.13$, $p = 0.72$; OGM: LR, $\chi^2 = 0.69$, $p = 0.41$; interaction: LR, $\chi^2 < 0.01$, $p = 0.99$), whereas offspring sired by *L*-males received significantly more food than nymphs sired by *S*-males in families where OGM and OFM mismatched (figure 2*a*; binomial GLM, $n = 81$; OGF: LR, $\chi^2 = 5.49$, $p = 0.019$; OGM: LR, $\chi^2 = 0.23$, $p = 0.63$; interaction: LR, $\chi^2 = 0.10$, $p = 0.76$). Second, interactions between effects inherited from genetic mothers and genetic fathers may determine if foster mothers or nymphs influence food provisioning, e.g. due to changes in offspring signals or behaviours. Consistent with this prediction, the origin of foster mothers significantly influenced provisioning when nymphs had mismatched maternal and paternal origins (figure 2*b*; binomial GLM, $n = 81$; OFM: LR, $\chi^2 = 6.69$, $p = 0.010$; OGF + OGM: LR, $\chi^2 = 0.67$, $p = 0.41$; interaction: LR, $\chi^2 = 0.15$, $p = 0.70$), whereas nymphs significantly influenced provisioning when they had matched parental origins (figure 2*b*; binomial GLM, $n = 80$; OFM: LR, $\chi^2 = 0.22$, $p = 0.64$; OGF + OGM: LR, $\chi^2 = 4.31$, $p = 0.038$; interaction: LR, $\chi^2 = 0.94$, $p = 0.33$).

(b) *Investment in second clutches*

The likelihood of second-clutch production by foster mothers was affected by the combined effects of OFM and OGF, as shown by a significant interaction between these two factors (table 1B). This result again supports the hypothesis of an entangled effect of co-adaptation and parental antagonism. Foster mothers were less likely to produce a second clutch when they tended nymphs sired by a male with different origin, regardless of the order of mismatching between OFM and OGF ($L + S$ or $S + L$; figure 2*c*), and the likelihood of second-clutch production was not additively inherited across generations (there were no significant main effects of OFM, OGM or OGF; table 1B). Furthermore, in line with the idea that family interactions and intrinsic female condition have independent effects on second-clutch production, we also found that the likelihood of second-clutch production was positively associated with the size of the first clutch produced by foster mothers (table 1B; mean ± s.e. first clutch size; 59.28 ± 2.62 and 65.64 ± 0.70 for one- and two-clutch females, respectively), a pattern previously suggested to reflect variation in female quality [22]. Finally, restricting the analysis to females that produced two clutches in the experiments, we found that their relative investment in second clutches was influenced by OGM. Foster mothers showed significantly larger relative investment when tending offspring produced by females from the *L*-group (number of eggs produced in second clutch divided by the total number of eggs produced: mean ± s.e. = 0.314 ± 0.009, $n = 70$) than from the *S*-group (0.292 ± 0.009, $n = 74$; table 1C). This relative investment was not significantly influenced by OFM, OGF or any of the interactions among factors (table 1C).

(c) *Offspring development and survival*

Our results revealed that interactions between OFM, OGM and OGF did not significantly influence the developmental time and the survival rate of offspring (table 1D,E), two traits not significantly correlated to

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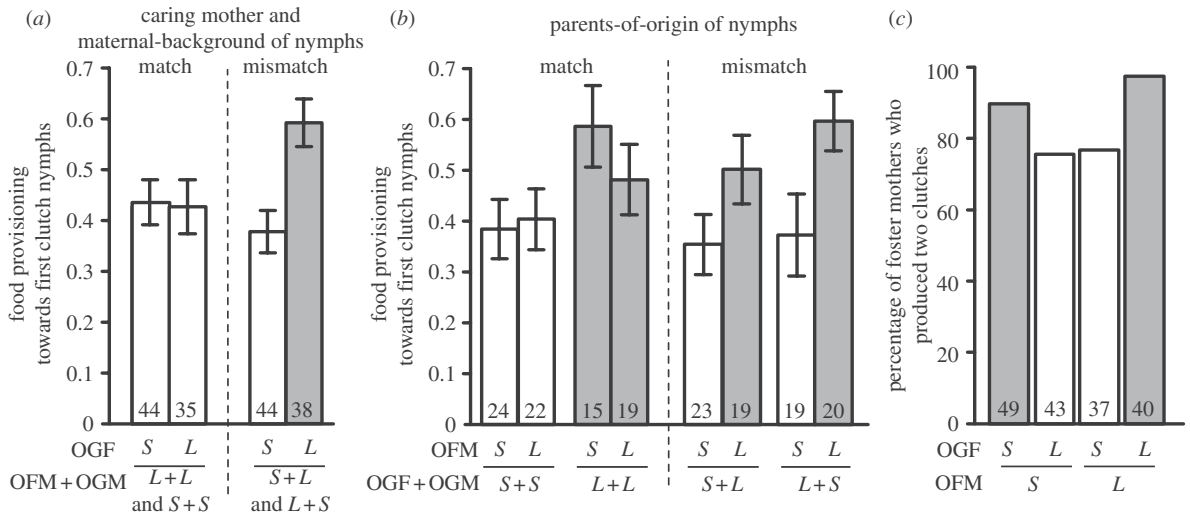

Figure 2. Food provisioning and likelihood of second clutch production by foster mothers. (*a*) OGF significantly influenced food provisioning in families where OFM and OGM mismatched, but not in families where OFM and OGM matched. (*b*) When nymphs had matched maternal and paternal genetic origins, nymphs with both *L*-origins received significantly more food than nymphs with both *S*-origins, independently from the origin of foster mothers. By contrast when nymphs had mismatched maternal and paternal genetic origins, nymphs from the two possible combinations received significantly more food when reared by foster mothers from *L*- than *S*-origins. (*c*) The proportion of females producing two clutches was significantly smaller when OFM and OGF mismatched than matched (*grey* versus *white* bars, $\chi^2 = 9.67$, $p = 0.002$). Error bars represent SEM in (*a*) and (*b*). Sample size is at the bottom of each bar.

each other (Spearman correlation test, $r_s = -0.13$, $p = 0.10$). Nevertheless, developmental time was significantly influenced by an interaction between OGF and clutch size (table 1D), with a positive correlation between developmental time and clutch size only in clutches sired by *S*-males (figure 3*a*); and offspring survival rate was significantly influenced by an interaction between OFM and clutch size (table 1E), due to a negative correlation between survival rate and clutch size only in clutches tended by *L*-foster mothers (figure 3*b*).

4. DISCUSSION

Our findings showed that post-natal interactions between mothers and offspring influenced maternal care and female future reproduction. This result confirms a little tested main assumption in all evolutionary models on parental antagonism, conflict resolution and co-adaptation [6,29], and reveals that offspring not only influence maternal care behaviours, but can also exert selection pressure on the caring females. Uniquely, we demonstrated that parental antagonism and co-adaptation have simultaneous and entangled effects on family interaction outcomes. In particular, we found that (i) the level of food provisioning reflected the combined influence of caring females, genetic mothers and genetic fathers of nymphs; (ii) the likelihood of second-clutch production by caring females resulted from the entangled effects of caring females and genetic fathers of nymphs; (iii) the relative investment of foster mothers in second clutch was shaped by the genetic mothers of nymphs, and finally that (iv) caring mothers and genetic fathers of nymphs independently influenced the developmental time and the survival rate of offspring.

We showed that parent-of-origin specific effects expressed in offspring mediated how nymphs influenced female traits (figure 2), a result predicted under parental antagonism and genomic imprinting [30]. Interestingly, however, we found that parental antagonism did not only occur through interactions between paternally and maternally inherited effects expressed in offspring (as predicted by the kinship theory of genomic imprinting [14–17]), but also through postnatal interactions between paternally inherited effects expressed in offspring and the caring females (a result in line with intergenomic social epistasis [7,25]). Such social epistasis between the caring female and the genetic father of offspring could generate correlational selection on these two family members [31], because matched origins of caring female and genetic father of nymphs (in terms of *S*- and *L*-groups) increased the female's likelihood of second-clutch production. If strong enough and sufficiently consistent over time and across environmental conditions, this correlational selection based on the resolution of family conflicts could favour assortative mating within populations and, ultimately, lead to speciation of individuals from *S*- and *L*-groups [32,33]. Interestingly, a previous study suggested that single and double-brooded earwigs (collected in separate populations) may correspond to two cryptic sister species in *F. auricularia* [34]. However, the support for this suggestion remains limited and our recent work demonstrates full mating compatibility between these two types of individuals (co-occurring within a same population) (see this study and [22]). Another possibility is that the populations remain panmictic, for instance, if no mechanism for assortative mating evolves. In this case, the occurrence of mismatched matings would contribute to the maintenance of variation in family interaction outcomes (as observed in the studied population [22]), thereby limiting long-term resolution of family conflicts. Disentangling these two evolutionary hypotheses requires tests for the presence of cues possibly

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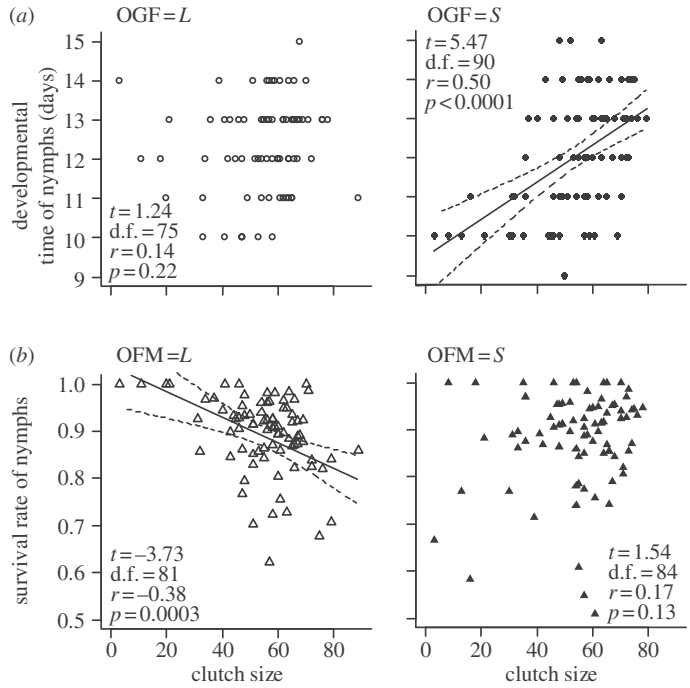

Figure 3. Developmental and survival rate of nymphs. (*a*) Nymph developmental time (number of days until the emergence of a second instars nymph in a clutch) was significantly sensitive to clutch size variation only when *S*-males sired the nymphs. (*b*) Nymph survival rate was significantly sensitive to clutch size variation only when *L*-mothers tended the clutches. Significant regression lines (filled lines) are shown with 95% confidence intervals (dashed lines). Pearson's correlation tests are reported.

involved in group recognition (e.g. chemical signatures [35]), as well as the occurrence of assortative mating and non-random fertilization between individuals from *S*- and *L*-groups.

Neither food provisioning nor second-clutch production was additively inherited from mothers to adult daughters. In particular, the non-significant main effect of OFM on these two traits measured on the females themselves indicates that the environment in which the foster mothers grew up as a nymph (and which is correlated with food provisioning and investment in second clutches, figure 1*a*) and/or the genes inherited from their parents did not predict (alone) second-clutch production and level of food provisioning (table 1A,B). Instead, our results reveal that the environment experienced by females and males as nymphs influences both the strategy they will later transmit to their own nymphs and how the newly produced mothers will react to such offspring strategy (in terms of food provisioning and second-clutch production). The above effects on female nymphs could be due to maternal effects transmitted to the eggs or to the young ones through family interactions, as reported in rats where pups experiencing low levels of care become mothers providing little care irrespective of their genotype [36]. Because fathers are absent during parental care, the above effect on male nymphs is likely to result from epigenetic sperm modification by early-life environments, for example, through induced mutations in DNA sequences, changes in the content of male ejaculate or epigenetic modifications in the male germline [37,38]. The molecular mechanism underlying the parent-of-origin specific effects reported in this study is currently unknown, but the observed patterns of inheritance are consistent with a role for genomic imprinting.

In contrast to the results on maternal traits, we found that cross-fostered offspring did not suffer from mismatched combinations of OFM, OGM or OGF in terms of developmental time and survival rate. The observed discrepancy in the effects of family mismatch on offspring and maternal traits suggests that parent–offspring co-evolution mediated by parental antagonism and co-adaptation is primarily driven by selection through the costs of care to females, rather than the benefits of care to offspring [4]. Nevertheless, we found that parents influenced alternative traits in offspring: genetic fathers influenced offspring sensitivity to clutch size in terms of developmental time (only the nymphs sired by *S*-males were sensitive), whereas foster mothers affected offspring sensitivity to clutch size in terms of survival rate (only the nymphs cared by *L*-females were sensitive). These parent-specific effects on two uncorrelated offspring traits reveal scope for antagonistic co-evolution between the sexes [19,39] over the control of offspring performance, in that a potential positive effect of fathers on offspring fitness (the developmental time of nymphs was not sensitive to clutch size when OGF was *L*) is counterbalanced by a potential negative effect of mothers (the survival rate of nymphs was sensitive to clutch size when OFM was *L*).

Over the past two decades, models of parental antagonism in animals mostly have been tested in placental species [9,13,17–19,40,41], although from a theoretical perspective selection for parent-of-origin specific effects applies more generally. By demonstrating parentally antagonistic effects in an insect species with basal and non-obligate forms of maternal care, our results

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emphasize that the evolution of parent-of-origin specific inheritance does not require intricate and obligate interactions between parents and offspring, for instance through a placenta.

In conclusion, our study demonstrates that parental antagonism and parent–offspring co-adaptation act as entangled key drivers of family interactions, even in species with facultative forms of care. This finding highlights the importance to consider these two major evolutionary processes together rather than in isolation to get a better understanding of the mechanisms regulating family interactions and promoting the evolution of social life [11,19,42]. Furthermore, our results demonstrate that the early-life social environment of offspring shape the strategy they later adopt as parents but also that they transmit to their own offspring. As a consequence the nature of mother–offspring interactions can be both cause and consequence of heritable variation in parental and offspring strategies (including the fitness of family members), providing an example for the importance of reciprocal causation in evolutionary biology.

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INVITED REVIEW

The evolution of parental care in insects: the roles of ecology, life history and the social environment

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Abstract. 1. Parental care increases the fitness of offspring at a cost to the parents in terms of residual reproductive success. This trade-off may be affected by ecology, life history and the social environment, which raises the question as to how these factors contribute to the evolution of parental care. Here, previous hypotheses concerning the evolution of parental care in insects are summarized and discussed and the underlying empirical evidence is reviewed.

2. Ecological factors such as harsh environments, ephemeral food sources or predation pressure are broadly accepted as evolutionary drivers of parental care. The most consistent evidence supports a role for natural enemies such as predators, microbes and cannibalistic conspecifics. Also, the importance of ecological factors may interact with the life history (parity) of a species, either as a pre-adaptation facilitating the evolution of parental care or as a consequence of enhanced parental investment under parental care. Yet, only limited experimental research has been carried out to test the combined influence of ecology and life history in the evolution of parental care.

3. Several forms of care can mediate the transition from solitary to family living, which entails the emergence of a novel – social – environment that generates new selection pressures from interactions within and between families. In this context, we review examples of studies on communal breeding, brood parasitism, parent–offspring conflict and co-adaptation, and discuss how these social interactions may in turn be influenced by ecological factors such as food availability or population density.

4. Insects are uniquely suitable for experimental and comparative research on the complex interplay between ecology, life history, and the social environment.

Key words. Benefits, co-adaptation, costs, environment, parental care.

Introduction

Parental care is considered a prime example for an altruistic trait that evolved to enhance the fitness of the recipients of care (offspring) at the expense to the donor of care (parents) (Royle *et al*., 2012). The costs of decreased parental residual reproductive success associated with parental care have to be outweighed by the parents' indirect benefit in terms of an increase in offspring fitness (Hamilton, 1964; Smiseth *et al*., 2012). This kin-selected indirect fitness benefit to the parents is typically associated with genetic conflicts between parents and offspring over the level of parental investment, because in sexually reproducing species, parents and offspring are genetically not identical (parent–offspring conflict; Trivers, 1974). There has been strong research emphasis on the importance of close genetic relatedness in the evolution of parental care, which resulted in a large number of theoretical and empirical studies (see Alonzo & Klug, 2012). The results of these studies are mixed, probably at least partly because the effect of kinship on the evolution of parental care also depends on variation between individuals and factors affecting the fitness benefits and costs of care, such as

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ecological conditions, the life history of individuals, conflicts between the sexes, and the social environment in which parents provide care (Alonzo & Klug, 2012).

Wilson (1975) proposed specific hypotheses about how ecological factors may influence the evolution of parental care. He predicted that parental care should predominately evolve under stable structured habitats, unusually stressful physical environments, high predation pressure, and scarce or specialized food sources. It was not until recently that the importance of ecological factors, in relation to the evolutionary origin of parental care, were rigorously investigated in a series of mathematical models. Klug and Bonsall (2010) showed that parental care can evolve from an ancestral state of no care under a range of combinations of ecological conditions and life histories (e.g. egg, juvenile, and adult mortality rates, adult reproductive rate, egg maturation rate, and the duration of the juvenile stage). The authors compared the evolution of parental care in a constant versus a variable environment. They found that in a variable environment, the selection of parental care depends on the interaction between environmental variability, the life-history traits affected by such variability, and the specific costs of care (Bonsall & Klug, 2011). For example, environmental variability reduces selection for parental care when the costs of care are associated with both reduced parental survival and reproductive rate, but favours parental care if the only cost of care is a reduced parental survival rate. Whereas recent theoretical developments support the idea that ecological agents of selection in combination with pre-existing life histories are important, they also revealed that ecological agents on their own are usually not sufficient for the emergence of parental care (Klug & Bonsall, 2010; Klug *et al*., 2012), leaving scope for other important factors. One of them is the social environment, which results from interactions between the two parents (Smiseth & Moore, 2004), between parents and offspring (Mas *et al*., 2009) or among siblings (Ohba *et al*., 2006). Such social interactions are indeed known to shape the benefits/costs ratio of care and, hence, possibly to influence the strength of natural selection on parental care once a basic level of care has evolved (Royle *et al*., 2002; Smiseth *et al*., 2012).

Our general aim in this review is to summarize and discuss hypotheses and empirical evidence from insects regarding influences of ecology, life history, and the social environments on the evolution of parental care. A great diversity in the forms of parental care has been reported across taxa (Tallamy & Wood, 1986; Clutton-Brock, 1991; see Royle *et al*., 2012 for a recent review). Besides birds and mammals, insects are a promising, albeit often understudied, system to investigate the evolution of parental care because it presents a particularly wide diversity in the forms, duration, and intensity of care (Trumbo, 2012) (see Fig. 1 for examples). Table 1 illustrates several well-studied examples of the variety of forms of parental care in non-eusocial insects and gives information about the sex of the caregiver.

Our review starts by discussing the empirical support for different ecological factors that favour the emergence of parental care. We pay particular attention to how ecological factors may interact with animal life histories (in particular semelparity versus iteroparity) and conclude that it remains unclear whether life histories are evolutionary causes or effects of parental care (or a combination of the two). We then elaborate on how the social environment can influence parental care via interactions within and between families. We discuss how family interactions can affect potential benefits and costs associated with parental care, and how parent and offspring strategies may evolve as a consequence of these socially mediated modifications of selection on parents and offspring. Finally, we discuss our perspective on areas of further research into the evolution of parental care and conclude that insects, with their broad diversity in extent and forms of care, offer a unique opportunity to conduct this kind of research.

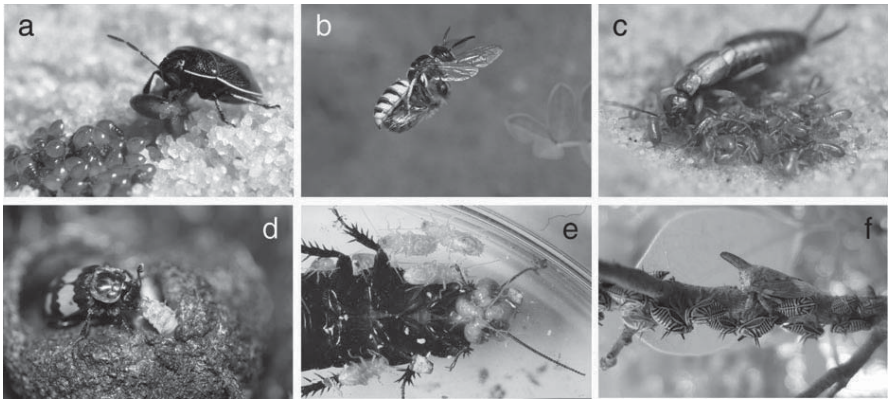

Fig. 1. A selection of insect species that provide parental care. (a) A female burrower bug (*Sehirus cinctus*) provisioning mint nutlets to her offspring (photograph: Patrick Alexander). (b) A female European beewolf (*Philanthus triangulum*) carrying a paralysed honeybee in flight to her nest (photograph: Gudrun Herzner). (c) A female of the European earwig (*Forficula auricularia*) with her first-instar nymphs (photograph: Joël Meunier). (d) A burying beetle *Nicrophorus vespilloides* providing food to its larvae via regurgitation (photograph: Per Smiseth). (e) Fourth-instar nymphs of the wood-feeding cockroach *Salganea taiwanensis* feeding on the stomodeal fluids of the female (view from below) (photograph: Kiyoto Maekawa). (f) A female treehopper (*Platycotis vittata*) with her brood of fourth- and fifth-instar offspring (photograph: Jennifer Hamel).

Table 1. Forms of parental care in insects. This table show a summary of well-studied and taxonomically diverse examples in which the benefits of parental care have been shown. Blank cells represent missing information.

Order/Family/species	Care giver	Form of parental care						References
BLATTODEA								
Blaberidae								
Blaberus craniifer	F		EB					Nalepa & Bell (1997)
Byrsotria fumigata	F		EB					Nalepa & Bell (1997)
Diploptera punctata	F			V				Roth & Willis (1957) Nalepa & Bell (1997)
Geoscapheus spp.	F		EB					Nalepa & Bell (1997)
Lanxoblatta emarginata	F				OA			van Baaren *et al*. (2003)
Macropanesthia spp.	F		EB					Nalepa & Bell (1997)
Nauphoeta cinera	F		EB					Nalepa & Bell (1997)
Neogeoscapheus spp.	F		EB					Nalepa & Bell (1997)
Parapanesthia spp.	F		EB					Nalepa & Bell (1997)
Perisphaerus spp.	F					OB		Roth (1981)
Phortioeca nimbata	F				OA			van Baaren *et al*. (2003)
Rhyparobia maderae	F		EB					Nalepa & Bell (1997)
Salganea spp.	B				OA		FP_2	Nalepa & Bell (1997); Maekawa *et al*. (2008)
Salganea taiwanensis	B						FP_2	Maekawa *et al*. (2008)
Schultesia lampyridiformis	F				OA			van Baaren *et al*. (2003)
Thanatophyllum akinetum	F				OA			Nalepa & Bell (1997); van Baaren *et al*. (2003)
Thorax porcellana	F					OB		Nalepa & Bell (1997)
Blattellidae								
Blattella germanica	F		EB					Roth & Willis (1957); Nalepa & Bell (1997)
Blattella vaga	F		EB					Roth & Willis (1957); Nalepa & Bell (1997)
Cryptoceridae								
Cryptocercus kyebangensis	B				OA		FP_2	Park *et al*. (2002)
Cryptocercus punctulatus	B				OA		FP_2	NI Seelinger & Seelinger (1983); Nalepa (1990)
Cryptocercus spp.	B	EA			OA		FP_2	Nalepa & Bell (1997); Maekawa *et al*. (2008)
COLEOPTERA								
Curculionidae								
Monarthrum spp.	F				OA		FP_1	Kirkendall *et al*. (1997)
Trypodendron lineatum	F				OA			Kirkendall *et al*. (1997)
Xyleborus spp.	F	EA			OA		FP	Kirkendall *et al*. (1997)
Passalidae								
All species	B				OA		FP_2	Schuster & Schuster (1997)
Scarabaeidae								
Onthophagus taurus	F						FP_1	Moczek (1998)
Silphidae								
Nicrophorus spp.	B				OA		FP_2	Scott (1990); Trumbo (1990)
Ptomascopus morio	F				OA			Trumbo *et al*. (2001); Suzuki & Nagano (2006)
Staphylinidae								
Bledius spectabilis	F	EA			OA		FP_2	Wyatt (1986)
DERMAPTERA								
Anisolabididae								
Anisolabis maritima	F	EA			OA		FP_2	Bennett (1904); Suzuki (2010)
Euborellia annulipes	F	EA			OA		FP_2	Rankin *et al*. (1995)
Euborellia plebeja	F	EA						Kamimura (2003)
Forficulidae								
Anechura bipunctata	F	EA			OA			Vancassel (1984)
Anechura harmandi	F	EA			OA		FP_3	Kohno (1997); Suzuki *et al*. (2005)
Forficula auricularia	F	EA			OA		FP_2	Weyrauch (1927); Lamb (1976a); Staerkle & Kölliker (2008)
Forficula decipiens	F	EA			OA			(M. Kölliker, unpublished)
Forficula lesnei	F	EA			OA		FP_2	Timmins (1995)
Labiduridae								
Labidura riparia	F	EA			OA		FP_2	Radl & Linsenmair (1991)
Spongiphoridae								
Chaetospania borneensis	F			V				Kocarek (2009)
Pygidicranidae								
Tagalina papua	F	EA			OA			Matzke & Klass (2005)

Table 1. Continued

Order/Family/species	S	Form of parental care				References
EMBIOPTERA						
Anisembiidae						
Anisembia texana	F	EA		OA		Choe (1994); Edgerly (1997)
Clothodidae						
Antiluparia urichi	F	EA		OA		Edgerly (1997)
Oligotomidae						
Oligotoma humbertiana	F	EA				Edgerly (1997)
HEMIPTERA						
Acanthosomatidae						
Elasmucha ferrugata	F	EA		OA		Kaitala & Mappes (1997)
Elasmucha fieberi	F	EA		OA		Melber & Schmidt (1975); Kaitala & Mappes (1997)
Elasmucha grisea	F	EA		OA		Melber & Schmidt (1975); Kaitala & Mappes (1997)
Belostomatidae						
All sp Belostomatinae	M		EB			Smith (1997); Estévez & Ribeiro (2011)
All sp Lethocerinae	M	EA				Smith (1997); Estévez & Ribeiro (2011)
Cydnidae						
Adomerus triguttulus	F	EA		OA	FP_2	Nakahira (1994)
Canthophorus niveimarginatus	F	EA			FP_2	Filippi *et al*. (2008)
Parastrachia japonensis	F	EA		OA	FP_2	Filippi-Tsukamoto *et al*. (1995b); Hironaka *et al*. (2005)
Sehirus cinctus	F	EA		OA	FP_2	Sites & McPherson (1982); Kight (1997)
Membracidae						
Polyglypta dispar	F	EA		OA		Eberhard (1986)
Publilia concava	F	EA		OA		Bristow (1983); Zink (2003b, 2005)
Publilia reticulata	F	EA		OA		Bristow (1983)
Pyrgauchenia tristaniopsis	F	EA				Stegmann & Linsenmair (2002)
Umbonia crassicornis	F			OA		Cocroft (1996)
Reduviidae						
Rhinocoris carmelita	F	EA				Thomas & Manica (2005)
Rhinocoris tristis	M/F	EA				Beal & Tallamy (2006)
Tingidae						
Gargaphia solani	F	EA		OA		Tallamy & Denno (1981)
Leptobyrsa decora	F	EA		OA		Loeb & Bell (2006)
HYMENOPTERA						
Bethylidae						
Goniozus nephantidis	F	EA		OA		Hardy & Blackburn (1991)
Megachilidae						
Osmia lignaria	F				FP_1	Torchio & Tepedino (1980)
Sphecidae						
Ammophila aureonotata	F				FP_1	Evans (1959)
Ammophila harti	F				FP_2	Evans (1959)
Ammophila juncea	F				FP_1	Evans (1959)
Ammophila nigricans	F				FP_1	Evans (1959)
Ammophila placida	F				FP_1	Evans (1959)
Ammophila procera	F				FP_1	Evans (1959)
Ammophila pubescens	F				FP_2	Evans (1959); Field & Brace (2004)
Ammophila sabulosa	F				FP_1	Field (1989)
Philanthus triangulum	F				FP_1	Strohm & Linsenmair (2001); Herzner & Strohm (2007)
ORTHOPTERA						
Gryllidae						
Anurogryllus muticus	F	EA		OA	FP_2	West & Alexander (1963)

B = biparental; EA = egg attendance; EB = egg brooding; F = female; FP = food provisioning, FP_1 = mass provisioning, FP_2 = progressive provisioning, FP_3 = matriphagy; M = male; NI = care after nutritional independence; OA = offspring attendance; OB = offspring brooding; V = viviparity.

Throughout this review, we follow the definition of parental care by Royle *et al*. (2012) as 'any parental trait that enhances the fitness of a parent's offspring, and that is likely to have originated and/or is currently maintained for this function'. Because we are interested in parental care *per se*, we decided to not include eusocial insects (e.g. Isoptera, Hymenoptera) in this review, because maternal care (i.e. from the queen to the brood) is commonly expressed only relatively briefly during colony foundation (Bourke & Franks, 1995; Queller & Strassmann, 1998; Boomsma, 2009). We limit our discussion to the evolution of parental care *per se* without addressing why it was often female uniparental care, instead of male uniparental or biparental care, that evolved. We correspondingly provide examples from these different modes of care without discussing selection on male versus female parental care, which was previously discussed, for example, in Tallamy (2001) and Trumbo (2012). For excellent former reviews on parental care in invertebrates (including insects as well) and on general social living in non-eusocial insects, we refer the interested reader to Trumbo (2012); Tallamy & Wood (1986) and Costa (2006), respectively.

Ecology, life history and insect parental care

In the following section we will explore previously proposed hypotheses for how ecological factors and variation in life history shape the evolution of parental care in insects. To this end, we first describe how ecological agents of selection are theoretically related to different forms of care, as hypothesized by Wilson (1971, 1975) and illustrate the evidence and its limits across insect taxa. Although the different ecological factors, in reality, probably rarely operate in isolation, we discuss them as separate, albeit not mutually exclusive, hypotheses for ecological factors that favour the evolution of parental care (Wilson, 1975).

Do harsh environmental conditions drive the evolution of insect parental care?

Whereas adaptations increasing egg development under harsh environmental conditions, such as heat stress, desiccation or high humidity, may include protection of the eggs themselves (e.g. a resistant egg shell), parental egg attendance provides an alternative route for resisting these factors. Attendance is expected to be superior to direct adaptations by the eggs if the parent suffers substantially less from the challenging condition than the eggs and/or the cost of the protective adaptation is higher than the cost of attendance for parents (i.e. the costs of egg attendance to the parents are exceeded by the benefits to the eggs). An added benefit of adaptation through parental care is that a caring parent can flexibly adjust its caring behaviour when necessary, whereas a resistant egg shell would be a fixed trait (see, e.g. Field & Brace, 2004).

Several studies provide direct or indirect empirical support for this hypothesis by reporting the benefits of maternal care under specific physical environmental constraints. For example, females of the terrestrial staphylinid beetle *Bledius spectabilis* live in the inhospitable habitat of the intertidal saltmarsh, wherein their burrows experience daily floods by the tide (Wyatt, 1986). To prevent flooding of their nest and anoxia of their eggs, females provide care in the form of closing the entrance of their burrow during high tide and reopening it at low tide (the latter being vital for respiration in the anaerobic soil). In the shield bug *Parastrachia japonensis* or the European earwig (*Forficula auricularia*), females attend their eggs and move them to a new nest site, if the physical conditions become unfavourable due to flood or desiccation (Weyrauch, 1927; Filippi-Tsukamoto *et al*., 1995a). Male belostomatid water bugs like *Belostoma flumineum* engage in brooding behaviour by keeping eggs wet, frequently exposing them to atmospheric air, and maintaining an intermittent flow of water over them by stroking them with the hind legs (Smith, 1976; Estévez & Ribeiro, 2011). If eggs become detached from the males, they fail to hatch. An extreme form of care that may occur under very low food availability is matriphagy. In the hump earwig (*Anechura harmandi*), an obligatory matriphagous species, first-instar nymphs kill and eat their mother before dispersing from the nest (Kohno, 1997; Suzuki *et al*., 2005). Hump earwig mothers do not seem to attempt escape from cannibalism by their nymphs and even do not produce a second clutch when being experimentally isolated from their nymphs. Thus, matriphagy provides important benefits to the offspring while the costs for the female seem very low due to the low chances of future reproduction (Suzuki *et al*., 2005). Also, anatomical/morphological adaptations by parents may enhance offspring fitness under harsh physical conditions. For instance, the brood sac of lecithotrophic and matrotrophic viviparous cockroaches such as *Rhyparobia maderae* or *Diploptera punctata* protects the developing offspring from heat, cold, moisture, desiccation, anoxia, and osmotic stress within the female body (Nalepa & Bell, 1997).

In these examples, it seems likely that harsh environments contributed to the described parental adaptations. Nevertheless, harsh conditions do not necessarily favour the evolution of parental care, because not only do they usually increase the potential benefits of parental care to offspring, but they may also induce parent–offspring competition for limited resources or enhance the costs to the parents to provide care under such aggravated conditions. Irrespective of the type of ecological harshness, it generally holds that if the costs of care exceed the associated benefits, care will not be selected for despite the potentially large benefits for the offspring (Clutton-Brock, 1991; Royle *et al*., 2012). Based on available data, it is currently difficult to judge whether the limited support is due to the limited cases in which parental care actually evolved under such conditions (providing evidence against evolution of parental care under harsh conditions), or to the limited amount of systematic research. Even if identified, a phylogenetic association between parental care and harsh environments does not prove that parental care evolved in response to selection imposed by such environments. Instead, such an association may reflect that species that have evolved parental care for some reason unrelated to the harshness of the environment may be able to colonize habitats that otherwise

would be inhospitable to ancestral species without parental care. There is clearly a need for further research on the question of if, and how, harsh environmental conditions favour the evolution of parental care, which should involve a combination of phylogenetic analyses and manipulative experiments to test directly how environmental harshness affects selection on parental care (i.e. using fitness assays under different environments with and without care).

Do ephemeral or distant food sources and specialized foraging drive the evolution of insect parental care?

Parental care is expected to allow the offspring to obtain food resources indirectly through the provisioning parent when food sources are ephemeral and occur clumped in space or time, or if they are difficult to access or process (as is often the case in specialized foraging). A critical problem when offspring need access to ephemeral and rare food sources is the extent to which a suitable and safe site for the offspring (e.g. a burrow or nest) is spatially disconnected from the food sources required for energy uptake. If juveniles are less mobile than adults, a provisioning parent may be able to provide both sufficient food and safe shelter at sustainable cost, selecting for parental provisioning of the ephemeral food source. The co-evolution of parental food provisioning and egg/offspring attendance for protection against natural enemies was recently modelled by Gardner and Smiseth (2011). In this model, parental food provisioning evolved from offspring attendance only if parental food provisioning was more efficient than offspring self-feeding, which is more likely to apply when food resources are ephemeral or difficult to access or process. Therefore, the model is in line with the general argument that these environmental factors may be important for the evolution of food provisioning.

There are well-studied examples of food provisioning among insects where the species feed on ephemeral food sources and/or where the offspring are spatially disconnected from it. For example, females of the shield bug *P. japonensis* provision nymph-containing nests progressively with drupes of a single host tree, *Schoepfia jasminodora* (Olacaceae), distant from the nest (Filippi *et al*., 2000). Similarly in the burrower bug *Sehirus cinctus*, nymphs only eat seeds of a few plant species, in particular *Prunella vulgaris* (Labiaceae) and *Lamium purpureum* (Labiaceae), which are available for only a few weeks each spring, and mothers might be better in competing for this limited resource (Kight, 1997).

The cockroach *Cryptocercus punctulatus* is an example of a species where specialization for a food source may underlie the evolution of parental care. In this wood-feeding species, nymphs are not able to directly process wood. First- and second-instar nymphs feed on hindgut fluids of both parents. Such behaviour allows them to acquire endosymbionts (intestinal flagellata), which are necessary for cellulose digestion and, hence, for the maintenance of this specialized foraging behaviour (Seelinger & Seelinger, 1983; Nalepa & Bell, 1997). In wood-feeding passalid beetles, all stages must feed on the faeces of mature adults. Faeces comprise shredded, digested wood, inoculated with bacteria and fungi from the adult digestive tract (Schuster & Schuster, 1997). Both *Cryptocercus* cockroaches and Passalid beetles feed on specialized food sources, but it should be noted that they also inhabit rather stable and structured environments (inside deadwood), another ecological factor that was hypothesized to promote the evolution of parental care (see later). It seems likely that a combination of these two factors was ultimately responsible for the evolution of parental care in these species.

In some species, females produce trophic eggs, i.e. unfertilized eggs that are used by hatched offspring as food sources – as, for example, in the Hemipteran *Adomerus triguttulus* (Kudô & Nakahira, 2004). We refer the interested reader to Trumbo (2012) for a more detailed discussion of this form of care.

Food provisioning is also present in species with non-specialized foragers feeding on non-ephemeral food sources. For instance, the European earwig, *F. auricularia*, is omnivorous and offspring are only partly disconnected from the food source, since nymphs are able to self-forage independently from an early age (Lamb, 1976a,1976b; Kölliker & Vancassel, 2007). Still, female food provisioning occurs across the order Dermaptera (Costa, 2006).

Given the inconclusive qualitative evidence, the hypothesis that ephemeral food sources and specialized foraging enhance the evolution of parental care would need a full quantitative test. Such tests should take into account other ecological conditions experienced by the species, its life history, the nesting habit and the feeding habit of the species, because selection for parental care is most likely under the combined influences of multiple factors (i.e. survival costs; Bonsall & Klug, 2011; Trumbo, 2012), for example when safe nests cannot be built close to the food source (Gardner & Smiseth, 2011), and/or when the offspring survival without parental assistance (mainly pre-digestion) is low.

Do natural enemies (predators, parasitoids, parasites, microbes) drive the evolution of insect parental care?

Predation was suggested repeatedly to play an important role in the evolution of parental care (Wilson, 1975; Tallamy & Denno, 1981). Whereas this hypothesis was originally put forward with regard to predators, it also applies in principle to any other natural enemy that can specifically impose harm upon offspring, such as parasitoids (Field & Brace, 2004) or microbes competing with offspring for food resources (Rozen *et al*., 2008; see Trumbo, 2012 for a detailed discussion). Exposure to natural enemies, especially of eggs and juveniles, may select for parental care only if the parents suffer substantially less from their exposure than the offspring. Protection can occur through egg/offspring attendance but other protective adaptations, such as the ovipositor or the resistant egg shell, can provide alternatives to enhance offspring fitness under pressure from natural enemies (Zeh *et al*., 1989).

The benefits of maternal egg/offspring attendance on offspring survival have been broadly studied and received consistent empirical support across insect species. For example, in the shield bug genus *Elasmucha*, females shelter the eggs

and nymphs by covering them with their body and fanning their wings when attacked. Egg survival was reported to be very low without care (Melber & Schmidt, 1975; Kaitala & Mappes, 1997), mostly due to predation. Females of the lace bug *Gargaphia solani* also show maternal antipredator behaviour and remain with their progeny throughout all five nymphal instars (Tallamy & Denno, 1981). In the absence of predators, nymphs suffer no ill effects if raised without their mother, but when nymphs were experimentally orphaned under normal field conditions, only very low numbers survived to maturity due to predation (Tallamy & Denno, 1981). Such effects have also been described in a sister species, *Gargaphia tiliae* (Hardin & Tallamy, 1992). In the staphylinid beetle *B. spectabilis*, maternal egg and offspring attendance protects eggs and larvae from predatory beetles or parasitoid wasps (Wyatt & Foster, 1989a,1989b). In the treehopper *Publilia concava*, maternal egg attendance effectively keeps away predators and the eggs are substantially more susceptible to these predators than are adults. Females exhibit two alternative tactics: immediate abandonment after oviposition or egg attendance until and beyond hatching. Zink (2003a) showed that a female attending her eggs until hatching doubled her hatching success relative to a female that abandoned her eggs immediately after laying. However, in terms of lifetime reproductive success, the enhanced fitness of the tending females through higher offspring survival was balanced by the reduced lifetime number and size of their clutches. Thus, tending and non-tending females had roughly similar fitness, which could explain why the two alternative reproductive tactics are maintained in treehopper populations (Zink, 2003a).

These are examples for interspecific predation. But intraspecific predation (i.e. cannibalism) can also be an important agent of selection in predatory insect species. As an example, a recent study in the earwig *Anisolabis maritima* demonstrated experimentally that egg attendance by females protects the eggs from being cannibalized by conspecifics (Miller *et al*., 2011).

Field and Brace (2004) showed experimentally in *Ammophila* wasps how progressive provisioning females can significantly reduce the impact of parasitism by cuckoo flies (Diptera: Miltogramminae), a major natural enemy of wasps. The cuckoo flies deposit live maggots that kill the immature wasp and then eat the provisions. Only wasp mothers of the progressively provisioning species could intervene and remove the fly maggots, which was not possible for mothers of mass provisioning species. Thus, there was an added benefit of progressive provisioning beyond the provided food in terms of protection against a parasite.

Empirical support for the benefits of parental care against competing microbes has been found in several species. Infestation by microorganisms is known to decrease offspring fitness either by killing the larvae or by decreasing progeny size and reproductive success. In the European beewolf (*Philanthus triangulum*), females provision brood cells with paralysed honeybees as larval food. Because the brood is located in warm and humid cells, there is a high risk of microbial decomposition of the provisioned food. Preservation of prey is achieved by the maternal application of chemical secretions that reduce fungal growth (Strohm & Linsenmair, 2001; Herzner & Strohm, 2007). An analogous mechanism was recently described in the burying beetle *Nicrophorus vespilloides*, where parents obligatorily breed on carcasses of small vertebrates, and larvae face intense competition with microbes over the carcass. The study by Rozen *et al*. (2008) showed that parents apply substances (e.g. lysozyme) that inhibit microbial growth and, hence, protect offspring by limiting the development of microbes that would otherwise quickly degrade the quality of the food source. The study further showed that the parental antimicrobial care resulted in higher larval body mass and survival. In the European earwig (*F. auricularia*), females have been shown to groom their clutch of eggs, a behaviour that has been hypothesized to prevent fungal infections and the moulding of eggs in their underground nests (Weyrauch, 1927; Lamb, 1976a).

Overall, the evidence seems robust for benefits of parental care in species where offspring face high risks of suffering fitness losses due to natural enemies that specifically target offspring or the resources they need for development and survival. Whether the pressure exerted by natural enemies is sufficient to favour the emergence of parental care remains to be confirmed experimentally, for instance by following changes in the level of parental investment in families reared under high and low predation pressures (i.e. experimental evolution). Furthermore, studies could also compare the effect of egg- or juvenile-specific predators with that of general predators, which differentially affect the cost/benefit ratio of protection to the parents and therefore the strength of selection on pre- and postnatal care.

Do predictable environments and life-history variation drive the evolution of insect parental care?

The reason why stable predictable environments may favour the evolution of parental care is linked with life-history evolution. Wilson (1975) and Tallamy and Brown (1999) suggested two contrasting hypotheses regarding the evolution of parental care and the mode of parity. Wilson (1975) argued that when a species adapts to stable, predictable environments, K-selection for an iteroparous life history (i.e. multiple reproductive attempts) tends to prevail over r-selection for a semelparous life history (i.e. single reproductive attempt). Under K-selection, individuals are predicted to live longer and grow larger, and also to produce a smaller number of offspring over multiple reproductive attempts, each with a high reproductive value and correspondingly high levels of parental investment. Following this line of argumentation, parental care is expected to predominately evolve among iteroparous species due to the high expected fitness returns on parental investment when each offspring represents a substantial fraction of lifetime reproductive success (here referred to as 'iteroparity hypothesis'). Tallamy and Brown's (1999) alternative hypothesis makes the opposite prediction that parental care should evolve more readily in semelparous species, because of the low evolutionary cost of care to parents in terms of residual fitness. Under this hypothesis, iteroparous insects should provide either no parental care or less care than

related semelparous species. The 'iteroparity' and 'semelparity' hypotheses suggest that the emergence of parental care does not primarily result from ecological selection pressures, but instead from life-history pre-adaptations shaping the investment trade-off between current and future reproduction.

Qualitative comparisons have been carried out to test these two hypotheses with mixed results. Some studies provide support for the 'semelparity hypothesis'. For example, Stegmann and Linsenmair (2002) tested this hypothesis in the membracid *Pyrgauchenia tristaniopsis*. Here, females exhibit relatively basic forms of care (i.e. egg attendance only) associated with a moderate degree of iteroparity (37% females produced a second clutch), whereas other membracid species generally express more elaborate forms of maternal care (i.e. egg and offspring attendance) and are typically semelparous. The authors interpreted this result as consistent with the 'semelparity hypothesis', in that iteroparity was associated with lower levels of maternal care. In another study, Nagano and Suzuki (2008) compared maternal investment in future reproduction between two species of Nicrophorine beetles: *Nicrophorus quadripunctatus*, which displays more elaborate parental care (carcass preparation, offspring attendance and provisioning); and *Ptomascopus morio*, which displays simpler parental care (offspring attendance only). In contrast to predictions of the 'semelparity hypothesis', the authors found that *Nicrophorus quadripunctatus* can oviposit several times in one breeding season and that they regulate their clutch size more strictly than *P. morio*. For more conclusive comparative tests, studies are now needed that relate parental care to parity across more than two species. Any two compared species are likely to differ in many ways that may also affect parental care (e.g. also ecology), which may confound the relationship and mask present patterns. Provided adaptive associations between life history and parental care exist, a different approach to test these hypotheses can be the comparison within species or within populations between individuals with different parity. Meunier *et al*. (2012) tested the association between the levels of maternal care and second clutch production in a population of the European earwig, *F. auricularia*, where semelparous and iteroparous females coexist. Contrary to the 'semelparity hypothesis', their results showed that iteroparous females provided significantly higher levels of maternal care in terms of food provisioning. They also produced larger first clutches and a larger total number of eggs (first and second clutch combined) than semelparous ones. The study suggests that the intrinsic condition of earwig females plays a key role in the level of maternal care and investment in future reproduction, in that high-condition females can afford both being iteroparous and providing more care despite a likely underlying trade-off between current and future reproduction.

One potential reason for the mixed evidence for an association between mode of parity and parental care is that the distinction between evolutionary cause and effect of parental care in terms of life history remains ambiguous. Is maternal care the consequence of a semelparous life history (as suggested by the 'semelparity hypothesis'), or is semelparity the consequence of the costs of parental care (referred to as the 'cost-of-care hypothesis')? Both directions of effects are likely to occur at differing relative strength between species. The question of whether a particular parity is a life-history pre-adaptation favouring the evolution of parental care, or whether it is, instead, the consequence of evolved parental care and the associated costs in terms of parental residual reproductive value has, to our knowledge, not yet been tackled theoretically or empirically. This distinction could be resolved through comparative phylogenetic studies by reconstructing the ancestral state and following the gain and loss of parental care in association with changes in parity. In Fig. 2 we provide the three phylogenetic hypotheses for the evolutionary association of parental care and mode of parity in insects and explain the different possible scenarios.

To conclude, despite a wealth of descriptions of diverse forms of parental care across insect species that vary in life history and inhabit different ecological niches, only little research has directly tested how environmental factors and life-history variation affect the benefits and costs of care (see also Trumbo, 2012). As previously mentioned, more experimental studies are needed, as well as phylogenetic analyses that combine the potential effects of a species ecology and life history on the evolution of parental care. Such an approach would provide a clearer picture of the importance of each ecological factor in relation to the evolution of parental care, while correcting for phylogeny and taxon biases resulting from differences in research effort across taxa, (e.g. the broadly studied

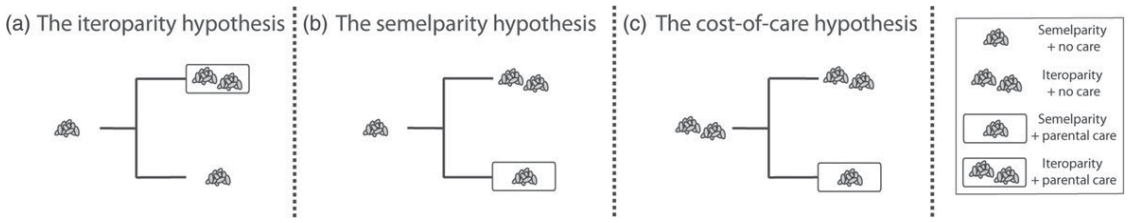

Fig. 2. Phylogenetic hypotheses for the evolutionary association of parental care and mode of parity in insects. In each panel (a)–(c), the ancestral state is depicted to the left of the tree, and the predicted derived states under each hypothesis to the right of the tree. (a) Wilson's 'iteroparity hypothesis' (1975): Wilson's hypothesis would be supported if care evolves in an iteroparous species as novelty from a semelparous ancestor, and no care remains associated with semelparity. (b) Tallamy and Brown's 'semelparity hypothesis' (1999): The 'semelparity hypothesis' would be supported if care evolved in a semelparous ancestor without care and iteroparous species derived from the same ancestor show no care. (c) The 'cost of care hypothesis': The hypothesis that semelparity is the consequence of a cost of care would be supported if care evolved in an iteroparous ancestor without care resulting in lineages where maternal care and semelparity co-occur as evolutionarily derived states.

cockroaches; see Table 1). To this end, some of the ecological parameters require standardized definitions (e.g. ephemeral food sources or harsh environments) and ways of measurement, in particular if we aim at comparative tests between insect taxa.

Social environment and the evolution of insect parental care

Parental care is typically associated with social interactions, such as those between parents and offspring or among siblings. The transition from solitary to group (family) living entails the emergence of a novel – social – environment that is characterized by the aggregation of parents and offspring, the resources provided by parents and the ensuing intensified social interactions among family members. This social environment forms part of an individual's ecology and generates new selection pressures, for example through selection on the effective transfer and usage of parentally provided resources, or through conflicts of interest within and between families (Trivers, 1974). Caring parents are a social environment to which offspring should adapt, and offspring are a social environment to which parents should adapt, and these novel selection pressures should lead to parent and offspring adaptations to family life and the co-adaptation of their traits (Kölliker *et al*., 2012).

When studying how the social environment influences costs and benefits of parental care, we have to consistently partition fitness components between parents and offspring (Wolf & Wade, 2001; see Smiseth *et al*., 2012 for a review). Parental care is beneficial to the offspring because it increases their direct fitness. From the perspective of the parent (or a parental care gene), the offspring fitness benefit is an indirect fitness benefit because the fitness of the genetically related recipient of care (i.e. the offspring) is enhanced, not that of the donor of care (i.e. the parent). Similarly, parents may pay a direct fitness cost of care in terms of their fecundity. From the perspective of the offspring, the parental fitness costs are indirect costs, because they are paid by genetically related individuals (i.e. the parents). These benefits to offspring and costs to parents lead to genetic conflicts over parental care (Trivers, 1974). It is expected that this selection favours mechanisms that contribute to a resolution of family conflicts, for example through the evolution of parent–offspring communication (reviewed in Kilner & Hinde, 2012). In insects, offspring influences on parental care have been shown to include vibrational signals (e.g. in treehoppers; Cocroft, 1996, 2001), tactile and visual begging (e.g. in the burying beetle *N. vespilloides*; Smiseth *et al*., 2003) and chemical signalling, that is solicitation pheromones (e.g. in the burrower bug *S. cinctus* and the earwig *F. auricularia*; Kölliker *et al*., 2006; Mas *et al*., 2009; Mas & Kölliker, 2011; see Mas & Kölliker, 2008 for review).

The reciprocal nature of parents and offspring influencing each other's fitness leads to selection on particular combinations of parental care and offspring traits, favouring co-adapted parent and offspring strategies. Co-adaptation models make two main predictions: first, that there is a genetic or epigenetic correlation between the levels of offspring demand and parental supply and, second, that a mismatch between parental and offspring strategies comes at a cost to family members (reviewed in Kölliker *et al*., 2012). Whereas these predictions were tested across numerous bird and mammalian species, parent–offspring co-adaptation has been explored in only three insect species so far. The first prediction of co-adaptation models was tested in the burrower bug *S. cinctus* and the burying beetle *N. vespilloides* using cross-fostering experiments, both providing evidence of a genetic correlation between maternal food provisioning and offspring begging (Agrawal *et al*., 2001; Lock *et al*., 2004). The second prediction of co-adaptation models was tested in *N. vespilloides* and in the European earwig *F. auricularia*. In *N. vespilloides*, offspring reared by foster females, i.e. in families with mismatched parental and offspring strategies, survived significantly less well than offspring reared by their own mother (Lock *et al*., 2004), and a recent study in *F. auricularia* demonstrated that earwig mothers caring for offspring with experimentally mismatched strategies suffered from fitness costs in terms of future reproduction (Meunier & Kölliker, 2012a).

Social environment and the costs of parental care

When multiple parents are breeding in close proximity, the potential network of social interactions is expanded beyond the core family (parents and offspring). Parents might interact with their own offspring, but also with other parents and their offspring. Such between-family interactions can be beneficial (in case of cooperative behaviours) or costly (in case of local competition for resources or brood parasitism). If the fitness or productivity of all individuals involved is increased simultaneously, we find a cooperative outcome due to direct benefits of communal breeding or brood mixing (Lin & Michener, 1972). Brood mixing can occur in species where offspring are mobile and can join other families. However, if an individual's expected reproductive output is even slightly decreased by the invading individual, the invader is more appropriately termed a parasite (Eberhard, 1986). In brood parasitism, one individual exploits the parental care invested by another individual. This could be through the female in case of egg dumping or through the offspring in case of brood mixing. Brood parasitic strategies are predicted to evolve, for example, when breeding sites are in close proximity and there is an opportunity for parental care to be misdirected. As a result, selection should favour kin recognition and guarding strategies in order for caring parents to avoid investment in foreign offspring, and offspring strategies to overcome such defense mechanisms in parents (reviewed in Keller, 1997).

Intraspecific brood parasitism was described in a number of insect species; for example, in the dung beetle *Onthophagus taurus* (Moczek & Cochrane, 2006), females use cow or horse dung to form brood balls that also serve as a food source for the larvae. Each brood ball contains a hollow chamber holding one egg. Females only oviposit one egg per brood ball, which constitutes the sole amount of food available for larvae to complete larval development (Moczek, 1998). Egg

dumping occurs as brood parasitic females were reported to replace conspecific eggs inside brood balls produced by another female with their own egg (Moczek & Cochrane, 2006). The authors suggested that the refilling of tunnels with previously excavated soil or sand by the caring parents is an adaptation to limit parasitism by conspecific females that makes it more difficult for other females to locate brood balls underground.

Parasitic strategies can also include social parasitism through the dispersal of mobile offspring invading foreign family groups. In the burrower bug *S. cinctus*, oviposition sites are aggregated in the field (Agrawal *et al*., 2004). The authors could not find evidence that neighbouring females were closely related, so brood mixing events could not have contributed to the females' inclusive fitness. Agrawal *et al*. reported that brood mixing occurred frequently in experimental studies, mainly initiated by nymphs under limited food supply. This could suggest that, under restricted food conditions, nymphs change their strategy from remaining with their own mother to dispersing and exploiting care from unrelated females, which could reflect brood parasitism. A field study by Kölliker and Vancassel (2007) showed that offspring of the European earwig *F. auricularia* dispersed from their own burrow and joined foreign family groups, and that this dispersal occurred more readily when the mother was removed (see also Kölliker, 2007).

In the case of intraspecific brood parasitism, conspecifics provide the only hosts for brood parasites and obligate parasitism cannot become fixed in a population. De Valpine and Eadie (2008) suggested that the advantages of egg dumping are likely to be greatest when the frequency of parasitism is low and many host nests are available containing few parasitic eggs. Thus, the parasitic strategy is expected to evolve under negative frequency-dependent selection, and the advantages will decrease as the frequency of parasitism increases and more host nests contain many parasitic eggs. As already pointed out by Müller *et al*. (1990), so far, we are unaware of cases of intraspecific brood parasitism in which individuals are restricted to either exclusive parasitic or non-parasitic behaviour. Intraspecific brood parasitism seems, rather, to be affected by environmental conditions such as population density (see the section on 'Ecological influences on social interactions' below) or the low likelihood for independent breeding by the parasitic individual.

Social environment and the benefits of parental care

Social interactions between families do not always result in parasitism. Sometimes both interacting sides can profit. For example, in cooperative breeders, some individuals postpone their personal reproduction in order to favour the reproduction of others, which was suggested to offer some of the strongest evidence of kin selection (Hamilton, 1964; Wilson, 1975). However, direct benefits such as communal territory defence, enhanced microclimate, enhanced foraging efficiency or nest/territory inheritance also favour the evolution of interactions between unrelated parents and between parents and offspring, including communal and cooperative breeding (e.g. Clutton-Brock, 2002; Bergmüller *et al*., 2007; Leadbeater *et al*., 2011).

Evidence of the direct benefits of communal breeding was found in a study on the parent bug *Elasmucha grisea*, a species where two females sometimes attend and defend their clutches jointly (Mappes *et al*., 1995). In a field experiment, Mappes *et al*. showed that communally guarding females had more eggs in their clutches than singly guarding females. The authors then confirmed this result in the laboratory by showing that joint unrelated females lost fewer eggs to ant predation than did single females, possibly because egg attendance is more than twice as effective with two females. The benefits of communal breeding are less clear in other species. In the burying beetle *Nicrophorus defodiens*, Eggert and Sakaluk (2000) showed that the presence of two females on a carcass did not reduce the risk of losing the carcass to other burying beetles. Scott (1994) suggested that communal breeding in the closely related *Nicrophorus tomentosus* reduces competition for carrion by fly maggots, a hypothesis that was later rejected by Eggert and Sakaluk (2000), who argued that large carrion flies cannot access carcasses once they are buried.

With regard to egg dumping (see earlier discussion; Tallamy, 2005), what appears to be a parasitic behaviour that is costly for the apparently parasitized individual might, in some cases, be beneficial for the dumper and the carer. Loeb *et al*. (2000) showed that females of the lace bug *G. solani* preferentially dump their eggs with kin, and that recipients gain indirect fitness by accepting these eggs. In their first bout of reproduction, significantly more of their own offspring survived to maturity in their first clutch than did controls without egg dumping (Loeb, 2003), most likely due to the predator dilution effect. In this case, egg dumping does not appear to be a parasitic strategy, but rather provides direct and indirect benefits of alternative reproductive tactics among closely related individuals.

The potential for intraspecific cooperation between females of the membracid *Polyglypta dispar* was suggested by Eberhard (1986). Multiple females were reported to oviposit in the same egg mass, and females sometimes adopted abandoned egg masses. Some guarding females attempted to prevent the visitor from ovipositing, whereas other guarding females just stepped aside. Guarding is a reproductively costly behaviour, since it delays the time to the next oviposition. Eberhard (1986) suggested that the probability of high genetic relatedness, due to philopatry contributes to the tendency for females to adopt abandoned egg masses. Furthermore, guarding females might benefit from the additional eggs, which are oviposited at the periphery of the egg mass, and Eberhard proposed that the eggs in the centre might become less subject to parasitism by parasitic wasps.

Parental care and family interactions need to be beneficial on balance for the offspring and the parent in order to evolve. Selection through direct or indirect benefits could have contributed to the evolution of parental care even if it is not purely directed to own genetic offspring, as described above by some exemplary studies. Such benefits could also partly explain why non-eusocial insect parents only rarely show sophisticated kin recognition and nepotism.

Ecological influences on social interactions

Ecological factors are expected to continue to shape selection on parental care once parental care originated. The evolutionary costs of certain amounts of parental care depend on the ecological context in which care is expressed. For instance, resource limitation in the environment is expected to modify the optimal investment in offspring, affecting the amount of resources transferred by parents to their offspring.

For example, in the European earwig (*F. auricularia*), females adjust the amount and duration of parental care to their own nutritional condition (Wong & Kölliker, 2012), which at least partly reflects food availability in the environment. Females provided food to fewer nymphs and for a shorter period of time, if their access to food was limited. Furthermore, in a study with the same earwig species, Meunier and Kölliker (2012b) showed that attendance by mothers can also be costly for offspring. Under food restriction, the usual fitness benefits of maternal presence for offspring (Kölliker, 2007) turn into a net reduction of offspring survival. The study could rule out the possibility that this effect was due to brood size adjustment by the female through filial cannibalism, as reported in the burying beetle *N. vespilloides* (Bartlett, 1987). Instead, it suggests direct competition for food between the female and her offspring under these conditions where the offspring pay the costs.

If offspring are not fully dependent on their parents (i.e. species with facultative care), they might take an active role in determining their own social environment. For example, work on the burrower bug *S. cinctus* showed that clutch joining initiated by the nymphs was especially high under insufficient food conditions (Agrawal *et al*., 2004). However, the consequences of joining between unrelated individuals (e.g. the increase in competition) were not investigated further. It would be interesting to test if females or nymphs exhibit discrimination against foreign offspring. The potential direct benefits of an increase in group size could explain why mothers accept foreign nymphs.

An increase in group size can also lead to local resource depletion and thus modify population dynamics. For example, Evans (1988) suggested that an increase in population density could result in an increase in intraspecific brood parasitism. This higher density can lead to scarcity of resources, such as breeding sites. For example, vertebrate carcasses of suitable quality are probably a scarce, unpredictable resource for burying beetles. Females of *N. vespilloides* fight for the ownership of carcasses and larger females usually manage to monopolize the carcass (Müller *et al*., 1990). However, the smaller female might stay near the carcass to lay her eggs for which the winning female will provide care. The lower the chances of finding another carcass on which no larger female is present, the more it pays a small female to stay and try to parasitize the winner's brood rather than leave. This results in costs for the larger female. Since larvae hatching from the parasite's eggs consume part of the available carrion mass, the number of offspring from the caring female was reduced.

Overall, these studies provide examples of how ecological conditions like population density or food availability influence variation in condition or parent–offspring relatedness. Other ecological factors, such as natural enemies, climatic change and the abiotic and biotic properties of the environment, can also influence selection through the social environment, for example by facilitating (or hindering) social interactions within and between families. This can affect social and family interactions and might modify or even reverse the usual benefits of parental care and turn them into costs paid by the parents and/or offspring.

Outlook

In this review, we have looked at former hypotheses regarding how ecological factors can affect benefits and costs of different forms of parental care, and how their effect on the evolution of parental care is expected to depend on the pre-existing life history of the species. When considering the likely complex relationships between ecology, life history and parental care in insects (see also Costa, 2006; Bonsall & Klug, 2011; Trumbo, 2012), we pointed out that the distinction between cause and effect is a critical one. Do some life-history traits facilitate the evolution of parental care or do the costs of parental care that evolve under particular ecological conditions lead to certain life histories? The wide variety of literature available, some of which has been presented here, is still short of systematic experimental studies that disentangle cause and effect between life history and ecology and that directly test factors that contribute to the evolution of parental care. The empirical evidence presented in Table 1 shows an over-representation of certain orders, e.g. the Blattodea. The large amount of work already available in these orders, together with the increasingly detailed molecular phylogeny of taxa, should lead to further investigations on the relationship among ecology, life history and phylogeny in the evolution of parental care.

To date, few studies have used comparative approaches to study the evolutionary history of parental care (Trumbo, 2012) but the following two are exemplary in demonstrating the scope that this approach has in answering evolutionary questions about the roles of ecology, life history, and the social environment in the evolution of parental care. Lin *et al*. (2004) used the molecular phylogeny of the treehopper subfamily Membracinae. Their results indicate that the ancestral state of the Membracinae is lack of maternal care and that there were three independent origins of egg attendance. The authors suggested that associated behaviours, life histories, and ecology may explain these origins, but the corresponding measurements were unfortunately not made. Gilbert and Manica (2010) went a step further and adopted a phylogenetic approach using quantitative data on body size, life history, and forms of care to test predictions about evolutionary associations between egg size, egg number (i.e. fecundity), and body size under different forms of parental care across 287 insect species from 16 orders. Their results showed that evolutionary changes in parental care were associated with lifetime fecundity rather than with egg size and that egg size was only influenced by body size (Gilbert & Manica, 2010). Such phylogenetic studies hold great promise to further our understanding of the evolutionary

origin of parental care when they are combined with close investigations and comparisons of the ecology, life history, and the social environment (Trumbo, 2012). This would comprise a large enough number of species, which show diversity in the forms of care within and among lineages as well as reliable measures of ecological factors and life history.

Besides phylogenetic work, more empirical studies investigating the evolution of parental care are required, and insects are probably uniquely suitable study systems to this end. Compared with mammals and birds that exhibit obligate forms of care, insects display a wide variability regarding the presence or absence of different forms of care, and regarding the degree of offspring dependence on these forms of care. Nevertheless, detailed experimental research on parental care and social interactions within and between families has been limited to comparably few species. Besides the investigation of causes and consequences of parental care and social environments, there is still also a need for basic natural history work because our knowledge of the diversity in the forms and extent of care is still limited in many taxa (Trumbo, 2012). Finally, the typically shorter generation time of insects compared with other model systems, and their easier maintenance under laboratory conditions enable us to investigate life-history traits associated with divergent patterns of care between closely related species, as well as the effect of specific environmental factors (e.g. variation in predation pressure or food resources) on long-term changes in the form and strength of parental care. Thus, insects are a highly interesting and suitable system to address open key questions on the evolution of parental care.

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Mother and offspring fitness in an insect with maternal care: phenotypic trade-offs between egg number, egg mass and egg care

Lisa K Koch and Joël Meunier*

Abstract

Background: Oviparous females have three main options to increase their reproductive success: investing into egg number, egg mass and/or egg care. Although allocating resources to either of these three components is known to shape offspring number and size, potential trade-offs among them may have key impacts on maternal and offspring fitness. Here, we tested the occurrence of phenotypic trade-offs between egg number, egg mass and maternal expenditure on egg care in the European earwig, *Forficula auricularia*, an insect with pre- and post-hatching forms of maternal care. In particular, we used a series of laboratory observations and experiments to investigate whether these three components non-additively influenced offspring weight and number at hatching, and whether they were associated with potential costs to females in terms of future reproduction.

Results: We found negative associations between egg number and mass as well as between egg number and maternal expenditure on egg care. However, these trade-offs could only be detected after statistically correcting for female weight at egg laying. Hatchling number was not determined by single or additive effects among the three life-history traits, but instead by pairwise interactions among them. In particular, offspring number was positively associated with the number of eggs only in clutches receiving high maternal care or consisting of heavy eggs, and negatively associated with mean egg mass in clutches receiving low care. In contrast, offspring weight was positively associated with egg mass only. Finally, maternal expenditure on egg care reduced their future reproduction, but this effect was only detected when mothers were experimentally isolated from their offspring at egg hatching.

Conclusions: Overall, our study reveals simultaneous trade-offs between the number, mass and care of eggs. It also demonstrates that these factors interact in their impact on offspring production, and that maternal expenditure on egg care possibly shapes female future reproduction. These findings emphasize that studying reproductive success requires consideration of phenotypic trade-offs between egg-number, egg mass and egg care in oviparous species.

Keywords: Reproduction, Parental care, Egg cannibalism, Reciprocal causation, Cost, Insect, Earwig

Background

The quantity and quality of offspring at egg hatching are two major components of parent and offspring fitness in oviparous species. Because these two components are traditionally thought to trade-off, females are expected to make optimal allocation decisions to maximize their reproductive success [1]. For instance, favoring the production of large clutch sizes (i.e. number of eggs) may increase the likelihood of getting a larger number of descendants. Alternatively, favoring the production of large (or massive) eggs may give rise to large offspring, which are better competitors and yield higher reproductive success than small ones [2]. Finally, spending a substantial amount of energy in egg care may enhance egg development and hatching success and may thus favor the production of numerous and / or better quality offspring [3].

Associations among egg number, egg size and egg care are generally expected to result from limited resources and life-history constraints [1,4]. For instance, limited resources have been shown to impose a trade-off between egg quantity and quality, with some females investing in

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large clutches of light eggs and others in small clutches of heavy eggs [2,5]. The detection of such trade-offs, however, assumes that all females within a population allocate the same quantity of resources to reproduction, which is not necessarily the case when females differ in quality (e.g. age or size) or resource acquisition [6,7]. Conversely, life-history constraints have been suggested to select for a positive association between egg size and maternal egg care [8]. One hypothesis to explain such an association is that it would allow the parents to increase the time spent with their offspring in the safest developmental stage (assuming that offspring life is hazardous and that larger eggs develop slower; safe-harbor hypothesis [9]). An alternative hypothesis is that egg mortality increases with egg weight, e.g. due to oxygen limitation in aquatic environments [6], so that these eggs require pre-hatching forms of care to develop properly [10]. To date, the ultimate reasons for the evolution of a positive association between egg size and maternal egg care are controversial, with recent comparative analyses revealing that its strength is taxon specific [11-15] and when it does occur, that its underlying evolutionary drivers are unclear ([8,10,14] but see [12]).

Although the associations between egg quantity, quality, and parental care were studied in many species [2,5,8,10,12,14], it remains unclear if and how these three parameters simultaneously shape maternal and offspring fitness at egg hatching. In general, disentangling pre- and post-laying effects on fitness returns is important for our understanding of the evolution of reproductive strategies and life-history traits [1,16], as these effects might reflect the outcome of independent selection pressures or of co-adaption processes. For instance, investment into pre-hatching forms of care may either reflect predetermined strategies of females, or serve to compensate for limited investment in the quality or quantity of eggs [17]. Here, we addressed this issue in the European earwig, *Forficula auricularia*, an insect species where females tend their clutch of eggs over winter and provide multiple forms of care, such as egg guarding, grooming and clutch displacement [18-20]. Whereas maternal attendance is required to ensure egg hatching, the frequency and duration of egg care (and thus maternal expenditure on these forms of care) are extremely variable among females [20-22]. Because earwig mothers stop feeding between egg laying and hatching [21], any change in female weight during this period can be used as a proxy to estimate its expenditure on pre-hatching forms of care. In particular, relatively high weight loss in females can be used to define high levels of maternal expenditure on egg care, whereas relatively low female weight loss (including weight gain due to egg consumption, see results) can be used to reflect low maternal expenditure on egg care. Interestingly, maternal care is not only shown towards the eggs, but also towards the young offspring (called nymphs) after hatching. In particular, mothers stay with their nymphs for several weeks during which they provide multiple forms of care, such as protection against predators and food provisioning [18,19]. Although post-hatching care is known to come with substantial costs for the mothers - for example by delaying their 2nd clutch production [18] - the potential costs of pre-hatching care remain unknown in this species. Finally, the size of earwig offspring at hatching is particularly important, as it generally enhances nymph survival and limits the risks of cannibalism after brood mixing, a common phenomenon during which nymphs join unrelated clutches [23,24]. Once the period of family life has ended, mothers disperse and some produce a second and final clutch [19].

We investigated the associations between egg number, egg mass and maternal expenditure on egg care, as well as their simultaneous influence on maternal and offspring fitness at egg hatching. We first surveyed a total of 80 clutches to (1) determine the occurrence of trade-offs among these three parameters, (2) test whether variation in female condition (body weight) possibly masks these trade-offs and more generally (3) investigate whether clutch size, egg size and maternal expenditure on egg care additively or interactively combine to determine offspring number and weight at egg hatching. In the case of interactions, we predict that egg number only determines nymph number and weight when mothers also express high investment into egg care (i.e. higher weight loss between egg laying and egg hatching). We then set up a mother-removal experiment using 40 clutches to test whether (4) low maternal expenditure on egg care can be beneficial to the female, e.g. in terms of 2nd clutch production, and whether (5) these benefits remain significant after females interact with their hatched nymphs [18,19,22,25].

Results

The 1st clutches produced by the 80 females contained between 47 and 91 eggs, which weighed between 0.55 and 0.75 mg on average and hatched between 21 and 28 days after they had been laid (Figure 1). The relative weight change of females during the period of egg care was highly variable, ranging from a 22.2% loss to a 16.8% gain of the initial weight. Note that maternal weight gain likely reflects egg consumption, as females had no access to food during the period of egg care. Most females (53, i.e. 66.3%) lost weight during the period of egg care (Figure 1), so that weight loss was used as a reference for weight change in the rest of the study (i.e. positive weight change stands for female weight loss and negative values for weight gain). In the mother-removal experiment, 18 out of the 20 isolated and 15 out of the 20 non-isolated females

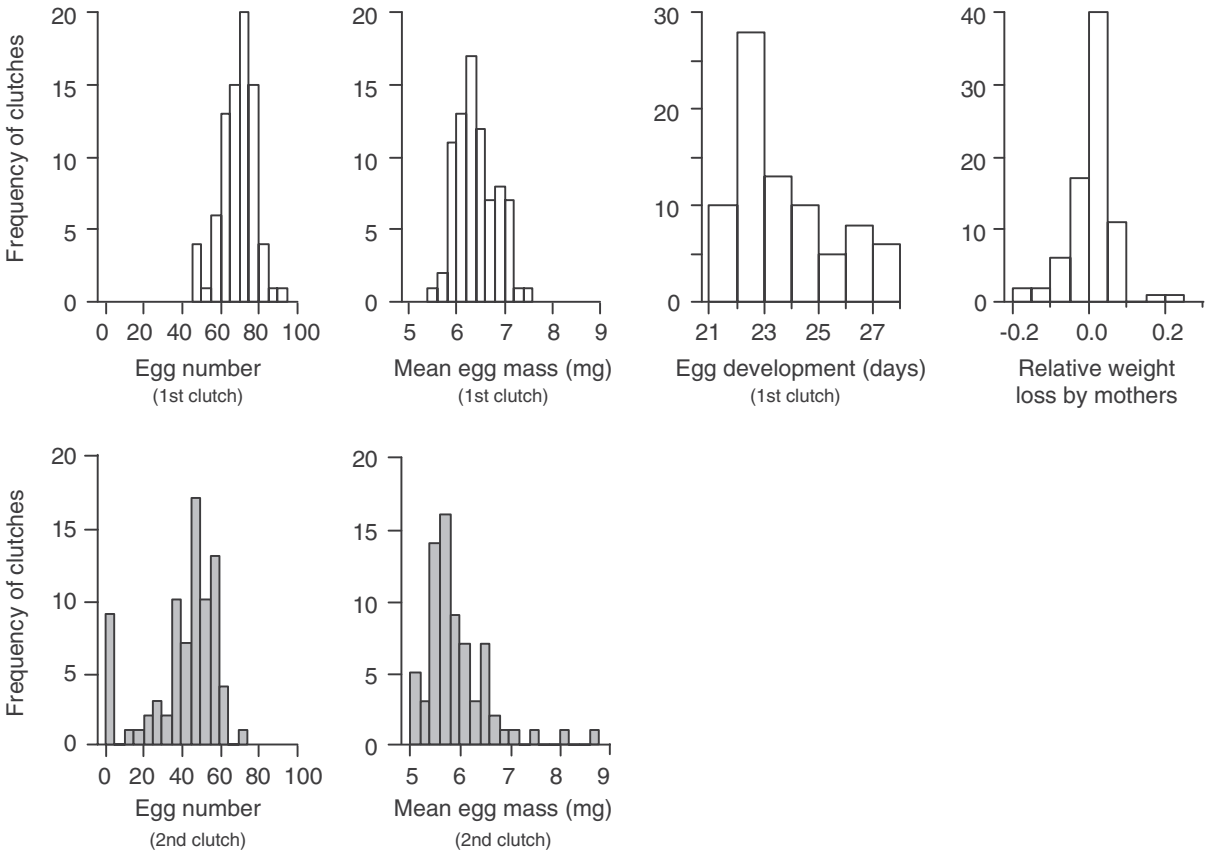

Figure 1 Distribution of the traits measured in first (white) and second (grey) clutches.

produced a 2nd clutch (Fisher Exact test, P = 0.408), which contained between 15 and 74 eggs, weighing between 0.50 and 0.87 mg on average (Figure 1). These 2nd clutches were overall smaller (paired t-test on egg number, t = 8.73, d.f. = 39, P < 0.0001) and lighter (paired t-test on mean egg mass, t = 2.65, d.f. = 32, P = 0.013) than the 1st ones.

Overall, there was no association between egg number, mean egg mass and the relative weight loss by mothers during the period of egg care (Table 1A). However, when correcting for variation in female weight at egg laying, the residuals of egg number were negatively correlated with both the residuals of mean egg mass (Table 1B) and the relative weight loss during egg care (Table 1B). Although heavy or large eggs are known to need more time to develop across species [9], we found that egg developmental time was independent of mean egg mass, of the number of eggs or of female expenditure on egg care (Table 1A and 1B).

A series of pairwise interactions among egg number, mean egg mass and female weight loss determined the number of hatched nymphs (Table 2A), a result supporting

Table 1 Associations of egg number, egg mass, maternal expenditure on egg care and egg developmental time

	(A) Uncorrected values				(B) Corrected values			
	Egg number	**Mean egg mass**	**Mother w loss**	**Egg dvpt**	**Egg number**	**Mean egg mass**	**Mother w loss**	**Egg dvpt**
Egg number	X	0.11	−0.20	−0.1	X	**−0.28**	**−0.24**	−0.01
Mean egg mass	0.321	X	−0.20	−0.08	**0.013**	X	−0.21	−0.05
Mother w loss	0.069	0.075	X	−0.09	**0.032**	0.066	X	−0.09
Egg dvpt	0.370	0.488	0.431	X	0.958	0.651	0.431	X

Maternal expenditure on egg care was estimated through the relative weight loss by mothers between egg laying and egg hatching. This correlation matrix was conducted using (A) uncorrected values or (B) the residuals of egg number and mean egg mass (i.e. values corrected for female weight at egg laying). The matrices above diagonal reports the correlation coefficient and the ones below diagonal the corresponding P-values, both obtained from Spearman rank correlation tests. The correlations significant after false discovery rate (FDR) corrections are in bold (see text).

Table 2 Influences of egg number, egg mass and maternal expenditure on egg care on (A) nymph number and (B) mean nymph weight

	(A) Nymph number			(B) Mean nymph weight		
	LR χ^2	d.f.	P	LR χ^2	d.f.	P
Egg number (EN)	56.07	1	**< 0.0001**	0.26	1	0.607
Mean egg mass (MEM)	0.05	1	0.816	8.78	1	**0.003**
Mothers weight loss (MWL)	7.78	1	**0.005**	0.35	1	0.552
EN : MEM	7.71	1	**0.005**	0.01	1	0.938
EN : MWL	11.66	1	**0.001**	1.18	1	0.277
MEM : MWL	7.27	1	**0.007**	0.01	1	0.921

Maternal expenditure on egg care was estimated through the relative weight loss by mothers between egg laying and egg hatching. Significant P-values are in bold.

the entangled effects of maternal expenditure on egg care and egg production on nymph production. In particular, decreases in mean egg mass or in relative weight loss by mothers during egg care cancelled the otherwise positive association between egg and nymph numbers (Table 2A, Figures 2A and 2B). Conversely, decreases in the weight loss by mothers entailed a negative association between mean egg mass and nymph number (Table 2A, Figure 2C). Independently from the interactive effects on nymph number presented above, the mean weight of nymphs at hatching was positively associated with the mean egg mass (Table 2B, Figure 3), but independent of egg number, mother weight loss or any interaction among the three tested factors (Table 2B). Overall, nymph number was independent of the mean weight of nymphs at hatching (Spearman rank correlation test; $r_s = -0.15$, S = 81049, P = 0.190).

The relative weight loss of mothers during egg care affected their investment into future reproduction, but this effect depended on the occurrence of post-hatching family life (GLM; Interaction between relative mother weight loss and occurrence of post-hatching family life: Likelihood ratio (LR) $\chi^2 = 11.33$, d.f. = 1, P = 0.0008). In particular, mother weight loss was negatively correlated with the number of 2nd clutch eggs when mothers were isolated from their 1st clutch nymphs at egg hatching (Figure 4; GLM estimate ± SE = −138.8 ± 73.4, t = −3.58, P = 0.001) but not when they were kept with their nymphs after hatching (GLM estimate ± SE = − 93.6 ± 80.8, t = 1.34, P = 0.188). In contrast, the mean egg mass of the 2nd clutch was independent of the three components of reproductive success measured on the 1st clutch and the occurrence of family life (GLM; Relative mother weight loss during 1st clutch: LR $\chi^2_1 = 0.30$, d.f. = 1, P = 0.583; Mean egg mass measured in the 1st clutch: LR $\chi^2_1 = 0.36$, d.f. = 1, P = 0.546; Post-hatching family life: LR $\chi^2_1 < 0.01$, d.f. = 1, P = 0.924; Interactions, all P > 0.384).

Discussion

Although egg number, egg mass and egg care are the three most common components of reproductive success in oviparous females, the reciprocal influences among them and the consequences of their joint action on maternal and offspring fitness remain under debate. Here we showed that in the European earwig *F. auricularia* (1) egg number, egg mass, expenditure on pre-hatching forms of care and egg developmental time are independent of each other. However, (2) when correcting egg mass and egg number for natural variation in female weight at egg laying, our data revealed a trade-off between these two parameters, as well as a trade-off between egg number and pre-hatching care. We also demonstrated that (3) the

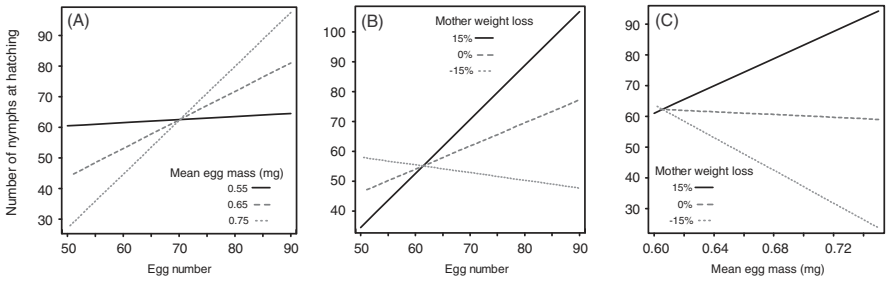

Figure 2 Interacting effects of egg number, egg mass and egg care on nymph number. The number of nymphs produced at hatching resulted from interactions between **(A)** egg number and mean egg mass, **(B)** egg number and mother weight loss and **(C)** mother weight loss and mean egg mass. As an illustration, the regressions lines are given for when **(A)** the mean egg mass was 0.55 mg (black), 0.65 mg (red) and 0.75 mg (green), as well as when **(B & C)** the relative female weight loss was 15% (black), 0% (red) or −15% (green).

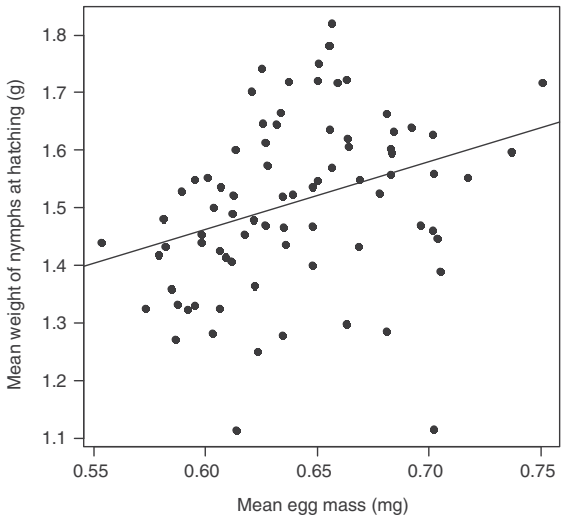

Figure 3 Correlation between mean egg mass and mean weight of nymphs at hatching.

number of hatching nymphs does not only depend on egg number, but also on the mean egg mass and on the level of maternal investment into egg care. In particular, low maternal expenditure on egg care led to a negative association between mean egg mass and nymph number, as well as weakened positive association between egg and nymph numbers. Moreover, clutches of light eggs were less likely to hatch than clutches of heavy ones. Independent of these effects on nymph number, our data showed that (4) the mean weight of nymphs at hatching was positively associated with the mean egg mass, but independent from egg number and investment into pre-hatching care. Finally, our results revealed that (5) maternal expenditure on the care of 1st clutch eggs reduced the production of 2nd clutch eggs, but only in absence of post-hatching family interactions.

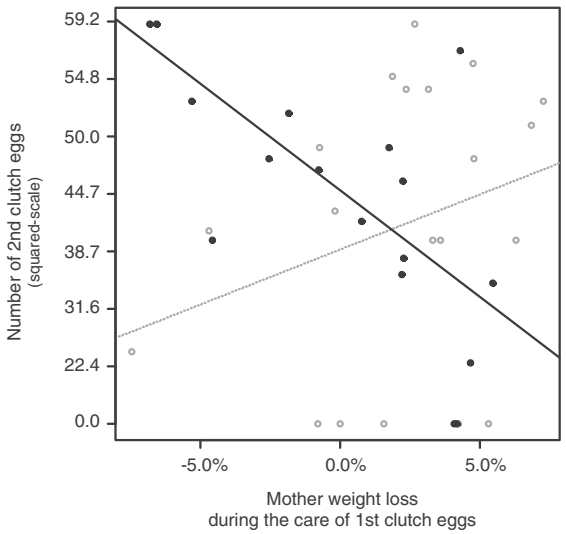

Figure 4 Influence of maternal expenditure on egg care on their 2nd clutch. Maternal expenditure on egg care was estimated through their relative weight loss between egg laying and egg hatching. After hatching of their 1st clutch eggs, females experienced (grey) or did not experience (black) post-hatching family life.

Variation in the amount of resources available to each individual is traditionally expected to mask potential investment trade-offs between mutually exclusive functions [26-28]. Our findings support this prediction in *F. auricularia*. Here, we show that correcting egg number and mean egg mass by female weight at egg laying allowed the detection of negative associations between egg quantity and quality and between egg quantity and egg care. The negative association between egg number and mean egg mass reflects a traditional trade-off found across a wide range of species [4,26-28], which shows females' needs to distribute resources among two traits simultaneously expressed at egg laying. Conversely, the negative association between egg number and egg care involves two temporally separated traits, which demonstrates that maternal expenditure on egg care is not a fixed strategy before egg production, but instead determined by females' clutch size. We propose four hypotheses to explain the reported level of maternal expenditure on pre-hatching forms of care. First, this level may simply be a by-product of the resources left to females directly after egg production, so that females are energetically constrained in their level of care [1]. The importance of resource availability on the level of (post-hatching) care, however, received mixed support across species (e.g. [19,29-31]). Second, it may reflect an adaptive strategy of the females which favors investment into either egg number or pre-hatching care [16]. For instance, low risks of egg predation and thus low benefits of egg guarding could favor females allocating more resources into egg number than egg care [32]. A third hypothesis is that the level of maternal expenditure on pre-hatching care might not be directly affected by egg production, but by the impact of egg production on egg quality. In other words, clutches where the number of eggs was higher than predicted by female weight could have required (or triggered) the expression of higher levels of care. Disentangling between these first three hypotheses would require cross-fostering of eggs and females [33], as well manipulation of clutch size to then investigate whether the level of maternal expenditure on egg care is determined by the number of eggs produced or tended by the female, as well as by the origin of the tended eggs.

The fourth hypothesis to explain the negative association between egg number and maternal expenditure in egg care is that larger egg production entailed larger egg consumption by females. Maternal expenditure on egg care was estimated by measuring mother weight loss

between egg laying and hatching, a period in which females had no access to food. Although this measurement may at least partly reflect energy expenditure [19,20], the fact that 27 (33.8%) females gained weight during this period of time implicates that at least some mothers consumed a few of their eggs. Filial egg consumption has never been reported in the European earwig (but it was seen in the maritime earwig, *Anisolabis maritima* [34]), but cannibalism frequently occurs in this species, either of eggs by newly hatched nymphs, between siblings during family life or even between adults during group living [21,35,36]. More generally, filial egg consumption is a behavior found in many species exhibiting parental care [37]. This behavior may reflects either (1) a stress-induced behavior with limited evolutionary relevance or (2) an adaptive strategy of females, which may serve to recycle resources from current eggs for future reproduction or into higher quality of care for the current clutch, as well as to limit the level of sibling competition after hatching [38-40]. In *F. auricularia*, the occurrence of filial egg consumption is unlikely to reflect a stress-induced behavior as all females were maintained under standard laboratory conditions that are typically associated with very high hatching success (e.g. [19,22,41]). Conversely, the benefits of low expenditure into pre-hatching care (and possibly of higher egg consumption) in terms of 2nd clutch production are in line with filial egg cannibalism as an adaptive strategy of earwig females. Further studies should investigate whether filial egg cannibalism results from the targeted consumption of non-viable (trophic eggs [3,42]) or viable eggs, and determine whether such behavior was selected to help females re-allocating resources toward future reproduction and/or limiting the level of future sibling competition.

Our data reveals both isolated and interactive effects of maternal expenditure on egg care, clutch size and egg weight on offspring weight and offspring number at egg hatching. On one hand, nymph weight was positively associated only with egg mass, a finding in line with those in other oviparous species [2,43]. On the other hand, nymph number was neither shaped by single nor additive effects among the three parameters, but by pairwise interactions. In particular, nymph number was positively associated with egg number only in clutches with heavy eggs or where mothers lost more weight during egg care, but also negatively associated with mean egg mass in clutches where mothers gained weight during egg care. If change in female weight at least partly reflects egg consumption, this negative association suggests that *F. auricularia* mothers preferentially fed on clutches of large eggs. Moreover, the overall negative association between mother weight gain and nymph number supports that female weight change during egg care is a good proxy to estimate the efficiency of maternal pre-hatching care (including the costs of egg consumption) on hatching success. Finally, the trade-off between egg mass and nymph number together with the positive association between egg mass and nymph weight reveals fitness costs and benefits of producing heavy eggs, but also shows that these costs can be limited when mothers subsequently invest into pre-hatching care (or limit egg consumption). Hence, maximizing fitness returns for earwig mothers and nymphs requires the simultaneous investments in heavy eggs and egg care. On the intra-species level, this finding is in line with comparative studies reporting the positive association between egg size and parental care across species and taxa [8,10-15]. More generally, it also supports that the sensitivity of heavy eggs to pre-hatching care could be a key driver in the emergence of such an association [12].

Lower weight loss (and weight gain) by mothers during the care of 1st clutch eggs translated into a higher number of eggs in the 2nd clutch, which revealed that low expenditure on egg-care (including high levels of egg consumption) provided benefits to females in terms of future reproduction. However, this association was only detectable when mothers were isolated from their hatchlings, suggesting that family life cancelled the maternal benefits of low expenditure on egg care and/or provided benefits to females with high expenditure on egg care, both in terms of 2nd clutch egg number. Whereas the second hypothesis requires forms of co-operation from nymphs to mothers, which to our knowledge have never been reported in species with family life, the first one could reflect a trade-off between pre- and post-hatching care, with females expressing low expenditure on egg care (including higher egg consumption rates) subsequently showing high levels of offspring care, and vice-versa. Because we did not measure the level of post-hatching care in this experiment, our data does not allow directly testing the occurrence of such a trade-off, or the ultimate reasons for its evolution (by-product or active strategy). However, this trade-off would be in line with the high variation in the levels of post-hatching care already described in *F. auricularia* females, e.g. in terms of food provisioning, aggressive protection against predators, clutch displacement and allogrooming [18,19,22,25]. More generally, this result raises the question of the importance of pre-hatching parameters and post-hatching family life on offspring fitness. For example, post-hatching maternal care has been shown to mask the otherwise positive effects of egg size on larval body mass at dispersal in the burying beetle *Nicrophorus vespilloides* [44]. Notice however that in the burying beetle females do not provide pre-hatching forms of care, which might have entailed different selection pressures on the respective importance of egg number and size, as well as pre- and post-hatching care between the two species.

Conclusions

In this study, we have shown phenotypic reproductive trade-offs between egg number, egg mass and pre-hatching care, as well as demonstrated their interactive effects on maternal reproductive success. We also demonstrated that studying natural variation in female body weight at egg laying is of key importance to better understand female investment trade-offs, as this variation masked the trade-off between the quantity and quality of eggs (a key trade-off reported in other species, e.g. [6,7,43]) and the one between egg quantity and maternal egg care. Moreover, our data suggests that pre-hatching care is costly for mothers in terms of future reproduction, but that these costs could be compensated by lower investment in post-hatching care. Overall, these results emphasize that studying fitness returns of oviparous mothers and offspring requires considering reciprocal influences among the multiple types of maternal investments at egg production, and more generally support the very recent claim to incorporate reciprocal causation in evolutionary theory [45].

Methods

A total of 80 females and 73 males of the European earwig, *F. auricularia*, were collected in September 2012 in Dolcedo, Italy. The individuals were then transferred to three plastic containers of comparable group size (balanced sex-ratio; 37 × 22 × 25 cm) and maintained for one month under standard laboratory conditions (12:12 h day:night, 20:18°C and constant 60% humidity). Each container was furnished with humid sand, egg cardboards and *ad libitum* food that was changed twice a week (see food composition in [19]). One month later, females were isolated in Petri dishes (10 cm diameter) to enable egg production [19]. The Petri dishes contained humid sand as a substrate, a plastic shelter as a nest and were maintained under complete darkness at 15°C and 60% humidity. Each female received *ad libitum* food until egg laying. Females were checked on a daily basis to record the first days of egg laying and egg hatching, and thus to calculate the egg developmental time (in days). Because eggs are generally laid within three days and hatch within one day, the number of eggs was counted three days after the first egg laying and the number of nymphs one day after the first egg hatching. On the days of counting, we weighed a group of ten randomly chosen eggs or ten randomly chosen nymphs per family and divided the values by 10 to obtain the mean egg mass and mean nymph weight, respectively. The relative weight loss by females during the period of egg care was measured by subtracting female weight at egg hatching from female weight at egg laying and dividing this value by female weight at egg laying. Maternal expenditure on egg care was defined as female weight loss during the period of egg care, because *F. auricularia* females (1) do not forage from egg laying to egg hatching [21], (2) lose weight due to their expression of energetically costly forms of care [19] and (3) may only gain weight due to filial egg consumption [34] so that negative female weight loss still reflects an extreme form of low expenditure on egg care. Note that all results remain qualitatively the same when using the absolute instead of the relative weight loss by females. All weighing was done to the nearest 0.001 mg using a micro scale (Pescale MYA 5).

We then investigated whether (1) the level of maternal care towards 1st clutch eggs affected their investment into 2nd clutch production and (2) whether post-hatching family life possibly masks such an association. To this end, 40 clutches were randomly sampled out of the 80 mentioned above (there was no difference between the two subsets regarding all the measured traits; MANOVA using egg number, mean egg mass, egg developmental time, number of nymphs at hatching, mean weight of nymphs at hatching, relative weight loss by mothers, mother weight at egg laying; Approx. $F_{7,72} = 0.91$, $P = 0.501$), while the other clutches were used in a different experiment (not presented here). Out of the 40 clutches, 20 mothers were isolated in new Petri dishes (diameter 10 cm) one day after their 1st clutch eggs hatched and 20 were first transferred to new Petri dishes with their 1st clutch nymphs for 16 days (under standard laboratory conditions, see [19]) and then isolated in new Petri dishes (diameter 10 cm). These two groups of 20 females did not differ regarding the above measured traits (MANOVA, Approx. $F_{7,32} = 0.82$, $P = 0.578$). Isolated mothers were then maintained under standard laboratory conditions (see above), received food twice a week and were checked on a daily basis to record 2nd clutch production. Seven out of the 40 females did not produce a 2nd clutch 60 days after their isolation and were thus considered as one clutch producers (i.e. the number of 2nd clutch eggs get the value 0, [19]). Like in the 1st clutch measurements, the number of eggs produced in the 2nd clutch and their mean mass were measured three days after the first egg has been observed. All experiments comply with European laws.

A series of spearman rank correlation tests was conducted to test for potential associations between the relative weight loss by mothers during egg care, egg number and mean egg mass. Because a key and common assumption in literature on maternal investment into egg quality is that large eggs take more time to develop [9], we also included egg developmental time in the correlation matrix. To determine whether female condition possibly masks trade-offs in the measured traits, we then re-ran the above correlation tests using the egg number and mean egg mass corrected for female weight at egg

laying. This correction was obtained by extracting the residuals from two linear models in which the female weight at egg laying was used as explanatory variable and either the egg number ($r = 0.54$, $F_{1,73} = 30.8$, $P < 0.0001$) or the mean egg mass ($r = 0.48$, $F_{1,73} = 21.6$, $P < 0.0001$) as response variable. The significance level $\alpha = 0.05$ in the correlation tests was adjusted for multiple testing to $\alpha = 0.033$ using the false discovery rate method [46]. Two Generalized Linear Models (GLM) were then used to test whether egg number, mean egg mass, relative weight loss by mothers and their interactions influenced the number and the mean weight of nymphs at hatching. Finally, two GLMs were fitted to test whether maternal expenditure on 1st clutch care influences female investment into future reproduction. A first GLM was run using the relative female weight loss during egg care, the number of eggs produced in the 1st clutch, the occurrence of post-hatching family life and their interactions as explanatory variables and the square-transformed number of eggs produced by females in their 2nd clutch as response variable. The second GLM was run using the same explanatory variables, but with the mean egg mass of the 2nd clutch eggs as response variable. All statistical analyses were conducted using the software *R* 3.0.2. Interactions between continuous factors were plotted using the package *effects*, which display the predicted values of a given GLM while controlling for the values in one of the interacting variables (details in [47]).

Availability of supporting data

The data set supporting the results of this article is available in the DRYAD repository, http://doi:10.5061/dryad.p9t05 [48].

Competing interests

The authors declare that they have no competing interests.

Author's contributions

LKK and JM conceived and designed the experiments. LKK carried out the experiments. JM conducted the statistical analyses and wrote the paper. All authors read and approved the final manuscript.

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Short-term benefits, but transgenerational costs of maternal loss in an insect with facultative maternal care

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A lack of parental care is generally assumed to entail substantial fitness costs for offspring that ultimately select for the maintenance of family life across generations. However, it is unknown whether these costs arise when parental care is facultative, thus questioning their fundamental importance in the early evolution of family life. Here, we investigated the short-term, long-term and transgenerational effects of maternal loss in the European earwig *Forficula auricularia*, an insect with facultative post-hatching maternal care. We showed that maternal loss did not influence the developmental time and survival rate of juveniles, but surprisingly yielded adults of larger body and forceps size, two traits associated with fitness benefits. In a cross-breeding/cross-fostering experiment, we then demonstrated that maternal loss impaired the expression of maternal care in adult offspring. Interestingly, the resulting transgenerational costs were not only mediated by the early-life experience of tending mothers, but also by inherited, parent-of-origin-specific effects expressed in juveniles. Orphaned females abandoned their juveniles for longer and fed them less than maternally-tended females, while foster mothers defended juveniles of orphaned females less well than juveniles of maternally-tended females. Overall, these findings reveal the key importance of transgenerational effects in the early evolution of family life.

1. Introduction

Family life is a common phenomenon in nature and is usually associated with substantial fitness benefits for offspring. These benefits mostly derive from the expression of parenting behaviours [1,2] such as nest construction, brood/juvenile attendance or food provisioning [2,3], and are thus contingent on the parental presence. Consequently, parental loss, e.g. due to clutch desertion or premature mortality, has been predicted to entail severe fitness costs for offspring that may ultimately select for the maintenance of family life across generations. In line with this prediction, short-term costs of parental loss have been reported in a large set of taxonomically diverse species, in which it is typically associated with a reduction in growth and/or survival rates of juveniles [1,4–6]. Importantly, other studies also showed that parental loss can entail long-term and transgenerational costs by hampering the mating success of adult offspring and diminishing the level of care they express towards their own descendants [1,7,8]. For instance in rats, females that had experienced long periods of maternal loss as pups exhibited low levels of care towards their own offspring, which in turn also exhibited lower levels of care as F_2 adults [9,10].

Somewhat surprisingly, the long-term and transgenerational effects of parental loss have only been studied in altricial vertebrates, in which juveniles exhibit limited foraging capabilities and thus heavily rely on parental resources [11–13]. However, investigating the occurrence of these effects in precocial invertebrates, in which juveniles exhibit early foraging capabilities and consequently only facultatively rely on parental resources [14,15], could provide crucial information on the early evolution of parental care. Indeed, transgenerational costs could be a key promoter of the maintenance of family life when parental loss has limited (if any)

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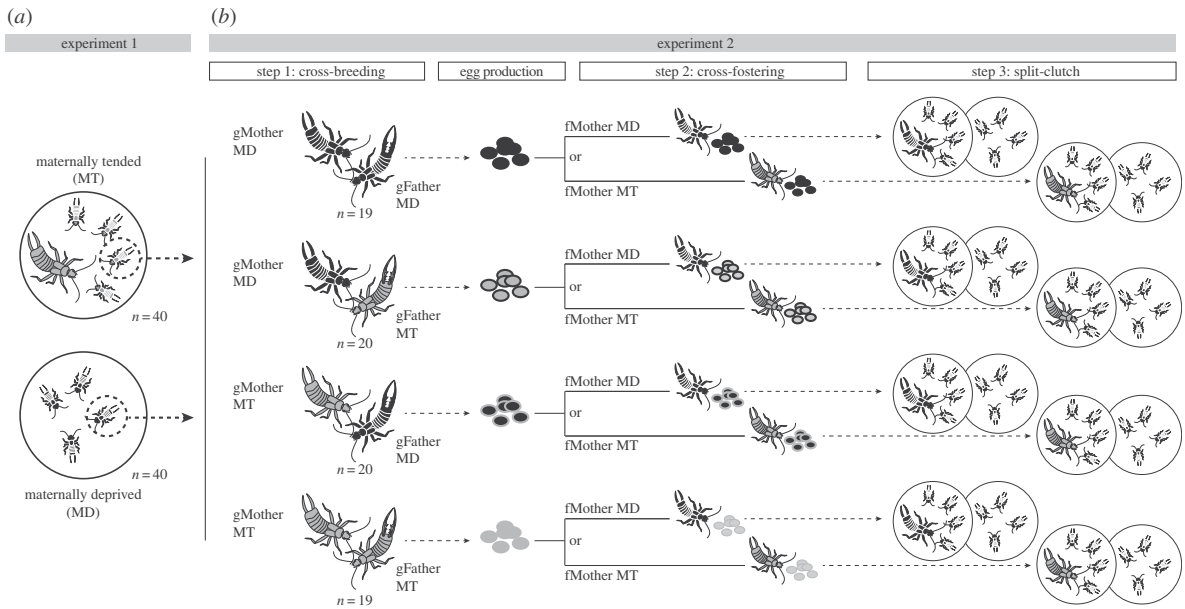

Figure 1. Experiments investigating the (*a*) short-term as well as (*b*) long-term and transgenerational effects of maternal loss. Transgenerational effects could be mediated by the experimental background of the genetic mother (gMother) and genetic father (gFather) of the offspring, as well as the one of their foster mother (fMother). Grey individuals have been maternally tended (MT), whereas black ones have been maternally deprived (MD).

short-term costs in terms of offspring survival, a scenario that applies to precocial systems and probably prevailed in the early evolution of family life [16]. Furthermore, studying the consequences of parental loss in precocial invertebrates could help to determine whether the tight association between parental care and offspring survival (as is found in altricial vertebrates) is a prerequisite for the expression of transgenerational costs, thus shedding light on the importance of these costs in the multiple forms of family life.

Here, we used a series of two experiments encompassing three generations of individuals to investigate the short-term, long-term and transgenerational effects of maternal loss in the European earwig *Forficula auricularia*. In this precocial insect, mothers provide care to their eggs over winter and, after hatching, to their mobile juveniles (called nymphs) [17,18]. Post-hatching maternal care lasts for several weeks and takes multiple forms including nymph grooming and food provisioning through regurgitation [19,20]. Although the early foraging capability of nymphs allows them to survive in the absence of a tending mother, maternal loss has been shown to entail short-term costs under suboptimal food quality, as it reduces the survival rate of nymphs until their fourth and last developmental instar [15]. Conversely, a second study showed that maternal loss enhances nymph survival rate when food quantity is limited, a result possibly due to a mother–offspring conflict over restricted food access [21].

In our first experiment, we investigated the short-term effects of maternal loss on the newly produced offspring. To this end, we reared nymphs with or without their mother under ad libitum food conditions and then monitored their development and survival rates until adulthood, as well as measured body size and forceps length in adults (two known fitness-related traits [22,23]). If maternal care was a key component of offspring development and survival, we would expect that maternally-deprived adults emerge earlier due to developmental stress [21], survive less well and exhibit smaller body and forceps sizes compared with maternally-tended adults. In our second experiment, we used the adults produced in the first experiment to determine whether maternal loss had long-term and transgenerational effects on the expression of maternal care. The expression of maternal care is generally known to reflect phenotypic and/or genetic traits of the caring mother, as well as maternally-inherited and paternally-inherited traits expressed by the tended juveniles, e.g. through maternal effects and epigenetic modifications [20,24,25]. We, therefore, conducted a full-factorial cross-breeding/cross-fostering experiment, in which we mated maternally-deprived and -tended females with maternally-deprived and -tended males, and cross-fostered the resulting eggs to a foster mother of the same or a different experimental group. We then measured the reproductive output of these families and determined the level of maternal care expressed by the foster mothers. If maternal loss had long-term negative effects, we would expect maternally-deprived mothers to exhibit a lower reproductive output and to express lower levels of maternal care. If maternal loss had transgenerational effects, we would expect the genetic origin of nymphs to affect the expression of care by foster mothers.

2. Material and methods

(a) Experiment 1: short-term effects of maternal loss

(i) Experimental design

The short-term effects of maternal loss were tested by rearing nymphs from 80 families of the European earwig *F. auricularia* with or without their mother. These families descended from 80 females and 73 males that were collected in a natural population in Dolcedo, Italy in September 2012 and kept under standard laboratory conditions throughout the experiments (details in [18]). Upon egg-laying, females were isolated in Petri dishes (9 cm diameter). One day after egg hatching, approximately 40 nymphs per brood (original brood size: mean $\pm$ s.e. $= 61.2 \pm 1.3$) were set up in a new Petri dish either with their mother (maternally tended, $n = 40$ families; mean brood size $= 39.6 \pm 0.29$) or without

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their mother (maternally deprived, $n = 40$ families; 39.3 ± 0.35; figure 1*a*). Fourteen days later, all tending mothers were removed from the maternally-tended groups to mimic natural family disruption [20]. Both maternally-deprived and -tended nymphs were subsequently transferred to larger Petri dishes (14 cm diameter) to allow their development until adulthood. At adult emergence, males and females of each family were separated to avoid inbreeding and ensure virginity [26]. All Petri dishes contained humid sand as ground material, a plastic tube as a shelter and an ad libitum amount of laboratory food, which mainly contained carrots, flower pollen and dry cat food (detailed composition in [17]).

(ii) Measurements of life-history traits in F_1 individuals

We measured the developmental time and survival rate of F_1 nymphs at each developmental instar, as well as the eye distance (a proxy of body size) and the forceps length of the resulting adults. Developmental time was defined as the day at which the first nymph within a brood was observed to moult into the next instar (newly moulted individuals stay whitish for one day), a measurement known to predict the developmental time for the whole brood [27]. Survival rates were measured for each developmental instar by counting the number of offspring alive three days after the first moulted individual had been observed. For the 1st instar, nymph survival was measured on day 10 after egg hatching. Finally, the average eye distance and forceps length of two haphazardly chosen male and female adults per family were measured to the nearest 0.001 cm using a camera coupled to a binocular microscope (Leica DFC425, Leica Microsystems Ltd., Heerbrugg, Switzerland) and the software Leica Application Suite 4.5.0. Note that one or two individuals were removed at each nymphal instar to conduct another experiment not presented here. These removed nymphs were excluded from survival rate calculations.

(b) Experiment 2: long-term and transgenerational effects of maternal loss

(i) Experimental design

The long-term and transgenerational effects of maternal loss were tested using three successive steps: (i) cross-breeding maternally-deprived and -tended adults, (ii) cross-fostering the resulting eggs to foster mothers of either the same or a different experimental background than their biological mother, and finally (iii) splitting the resulting families into two groups, one of which stayed with the foster mother, whereas the other was orphaned (figure 1*b*). The cross-breeding was conducted by pairing 78 females with 78 unrelated males to obtain 19–20 replicates of each of the four possible combinations of maternally-deprived and -tended adults (figure 1*b*; electronic supplementary material, table S1). These adults were haphazardly chosen among the ones obtained from experiment 1. Note that two families did not produce enough adults and could thus not be used in experiment 2. Mating pairs were maintained under standard laboratory conditions for two months, after which females were isolated to allow egg laying [21].

The resulting eggs were cross-fostered on average 5.7 ± 0.7 ($\pm$s.e.) days after they had been laid. The cross-fostering was conducted to obtain the 16 possible combinations of experimental backgrounds of the parents (maternally-deprived or -tended), i.e. by controlling for the background of the genetic mother (gMother) and the genetic father (gFather) of the transferred eggs, as well as for the background of the recipient mother (then called foster mother, fMother) and the male who was paired with the foster mother (fFather) (figure 1*b*). Females of the European earwig readily accept foreign eggs [20], thus allowing us to ensure that foster mothers were always unrelated to the eggs they tended. After cross-fostering, all foster females and their adopted eggs were kept under standard laboratory conditions until egg hatching. Note that six of the 78 females were excluded from the cross-fostering, because they either did not produce any eggs or produced them too early/late to conduct a cross-fostering (details in the electronic supplementary material).

Finally, the split-clutch was conducted to determine whether maternal attendance could mask the transgenerational effects of maternal loss on offspring life-history traits. One day after egg hatching, the nymphs of each of the cross-fostered families were attributed to two equally-sized groups: one group was tended by the foster mother for 14 days (then called maternal presence), whereas the other group was raised without a mother (then called maternal absence; figure 1*b*). At day 14 after egg hatching, the foster females were discarded from the experiment and the nymphs maintained under standard laboratory conditions until they reached adulthood.

(ii) Measurements of maternal care by F_1 adults and of life-history traits in F_2 individuals

We measured the long-term and transgenerational effects of maternal loss on the expression of three forms of maternal care (in the maternal-presence groups). The first form was (i) clutch defence, which reflected the females' willingness to protect their clutch of eggs or nymphs from predator attacks. Note that under natural conditions, mothers typically stay on or in the vicinity of their clutch to defend it against predators such as pseudo-scorpions and male earwigs (J. Meunier 2015, personal observation). It was measured on day 10 after egg-laying (egg defence) and on day 4 after hatching (nymph defence), by standardly poking each female on the pronotum with a glass capillary (one poke per second) and then recording the number of pokes required until she moved more than two body lengths away from her clutch. The second form of maternal care was (ii) clutch desertion, which showed how long females abandoned their clutch after being chased away by a simulated predator attack. Under natural conditions, temporary clutch abandonment can be costly for females, as it allows predators—including conspecifics—to consume the clutch of eggs or nymphs. It was measured by recording the time the female took to return to her clutch of eggs or nymphs after the end of the clutch defence measurement.

Finally, the third form of maternal care was (iii) food provisioning, which revealed how much food earwig mothers provided to their nymphs. In brief, the process involved four standard [17] and successive steps that started at day 6 after hatching. They consisted of: (i) food depriving mothers and nymphs for 24 h, (ii) isolating females for 1 h while offering them ad libitum green-coloured pollen pellets (Hoyer and DEKO BACK), (iii) reassembling each female with a standardized number of 15 (or all if less than 15 available) of her foster nymphs for 15 h, and finally (iv) calculating the proportion of nymphs with a green-coloured gut. This measurement was not conducted in clutches with less than five nymphs. The nymphs from the maternal-presence groups not used in these tests, as well as all nymphs from the maternal-absence groups, were isolated under the same conditions. Once the green-coloured nymphs had been counted, the maternal-presence group was reassembled and all groups were provided with ad libitum laboratory food and maintained under the standard conditions. Owing to the requirement of at least five surviving nymphs per clutch to reliably measure food provisioning and time constraints associated with the simultaneous measurements of multiple forms of maternal care across clutches, we used 61 (85%) of the 72 cross-fostered clutches to measure clutch defence, 59 (82%) to measure desertion time and 47 (65%) to measure food provisioning (sample sizes in the electronic supplementary material, table S1).

We also explored the transgenerational effects of maternal loss on three egg and four nymphal traits (in both maternal-presence

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and -absence groups). The three egg traits were measured on the first clutch produced by each female and consisted of the number, mean weight (per clutch) and hatching success of these eggs. The number of eggs was counted 3 days after the first egg had been observed. Their mean weight was measured at that time by weighing a random sample of 10 eggs to the nearest 0.1 μg. The hatching success was obtained by dividing the number of nymphs one day after hatching by the number of eggs transferred to the foster female. The four nymphal traits included their number, mean initial weight, survival rates, as well as developmental time until adulthood. One day after hatching, the number of nymphs was counted and a haphazard sample of 10 nymphs weighed to the nearest 0.1 μg. Nymph survival rate was measured by dividing the total number of adults that emerged from each type of group by the number of nymphs originally transferred into these groups. Finally, nymph developmental time was defined as the number of days between hatching and the emergence of the first adult in each of the two groups. All weighing was conducted using a micro-scale (PESCALE, MYA5).

(c) Statistical analyses

The short-term effects of maternal loss were analysed using a series of one generalized linear mixed model (GLMM) and three linear mixed models (LMMs). In the first models, nymph survival rate (GLMM) or nymph developmental time (LMM) at each instar was entered as the response variable, while maternal loss (bimodal; maternally-tended or -deprived, respectively MT or MD) and each developmental instar (continuous) were used as explanatory variables. The family identification (ID) was entered as a random effect into these two models, because each family group was measured at each instar. In the other models, the mean eye distance (LMM) or the forceps length corrected for body size (see the electronic supplementary material for calculation; LMM) was entered as the response variable, and maternal loss and sex as explanatory factors. The family ID was also entered as a random effect, because each family group provided values for males and females.

The long-term and transgenerational effects of maternal loss were analysed in another series of linear models (LMs), generalized linear models (GLMs) and LMMs. The first models were fitted using either nymph number (LM), nymph weight at hatching (LM) or food provisioning (measured as proportion of coloured recipient nymphs, GLM) as response variable, and gMother, gFather and fMother as explanatory factors. In the second set of models, egg number (LM), egg weight (LM) or hatching success (GLM) was entered as response variable, and gMother and gFather as explanatory factors. In the last models, the response variable was either clutch defence (log-transformed number of pokes withstood; LMM) or clutch desertion (log-transformed time away from clutch; LMM), and the explanatory factors were gMother, gFather, fMother and the type of clutch (eggs or nymphs). In these LMMs, the ID of the clutch was entered as a random effect, because the measurements were conducted on eggs and nymphs from the same clutches.

Finally, offspring developmental time and survival rate until adulthood were analysed in two separate steps. In the first step, the effects of gMother, gFather and fMother on offspring developmental time (LM) and survival rate (GLM) were tested in the split-clutches tended by a foster mother (i.e. in the maternal-presence groups only). Because fMother was never significant in these tests (electronic supplementary material, table S2), we pooled the two levels of this factor and, in a second step, compared offspring developmental time and survival rates of maternal-presence and -absence groups. To this end, we tested whether gMother, gFather and/or the presence of a tending mother (yes or no) affected either the developmental time (LMM) or the survival of nymphs until adulthood (GLMM). The ID of the original clutch was entered as a random effect into these two models because split-clutches with and without a tending mother were used.

Statistical analyses were conducted using the software R v. 3.1.3. All interactions between explanatory factors were tested in each model, which were then simplified stepwise by removing the non-significant interactions (all $p > 0.12$). All GLM(M)s were fitted with a binomial error structure corrected for overdispersion. Proportion data (e.g. survival and developmental rates) were entered into these models using the *cbind* function. Note that fFather was not entered into any statistical model because there was no specific prediction based on this effect for the different measurements (see also [20]) and this ensured an average of 7.5 replicates for each of the tested combinations (electronic supplementary material, table S1).

3. Results

(a) Experiment 1: short-term effects of maternal loss

Contrary to the predicted short-term costs of maternal loss, we found that maternal loss positively shaped two morphological traits in adult offspring (figure 2). Specifically, maternally-deprived adults had wider eye distances (figure 2*a*; Likelihood ratio (LR) $\chi^2_1 = 5.75$, $p = 0.016$) and longer forceps (corrected for eye distance; figure 2*b*; LR $\chi^2_1 = 6.83$, $p = 0.009$) than maternally-tended adults. Moreover, eye distance was overall wider for females (mean ± s.e. = 1.42 ± 0.006 mm) than for males (1.39 ± 0.006 mm; LR $\chi^2_1 = 8.25$, $p = 0.004$) and was not shaped by an interaction between maternal loss and sex (LR $\chi^2_1 = 1.25$, $p = 0.264$). The statistical model on the corrected forceps length reported no main significant effects of sex (LR $\chi^2_1 = 0.36$, $p = 0.547$) or of the interaction between maternal loss and sex (LR $\chi^2_1 = 3.58$, $p = 0.058$).

Maternal loss did not influence nymph survival rate (figure 2*c*; LR $\chi^2_1 = 0.05$, $p = 0.826$) and developmental time (figure 2*d*; LR $\chi^2_1 = 0.45$, $p = 0.502$) until adulthood. Not surprisingly, the nymphs survival rate decreased with time (LR $\chi^2_1 = 919.19$, $p < 0.0001$; model estimate ± s.e. = −0.81 ± 0.04), while the nymphs' developmental time increased with developmental instar (LR $\chi^2_1 = 6543.81$, $p < 0.0001$; estim. ± s.e. = 17.04 ± 0.30). However, nymph developmental instar did not interact with maternal loss to shape these two measurements (survival rate: LR $\chi^2_1 = 0.53$, $p = 0.465$; developmental time: LR $\chi^2_1 = 0.002$, $p = 0.963$).

(b) Experiment 2: transgenerational effects of maternal loss

Maternal loss affected the expression of three forms of maternal care by adult offspring through long-term effects in the caring mother, as well as through inherited, parent-of-origin-specific effects expressed in the nymphs (table 1). First, maternally-deprived foster mothers abandoned their clutch for an overall longer period of time than maternally-tended foster mothers (table 1*b* and figure 3*a*), an effect that was independent of the genetic parents of the offspring and of the age of the clutch (i.e. eggs or nymphs, table 1*b*). Second, the maternally-deprived foster mother provisioned overall less nymphs with food than maternally-tended foster mothers (table 1*c* and figure 3*b*). Food provisioning was also shaped by an interaction between the experimental backgrounds of the two genetic parents of the tended nymphs (table 1*c*). Nymphs of maternally-tended males received more food when they had been produced by maternally-deprived compared

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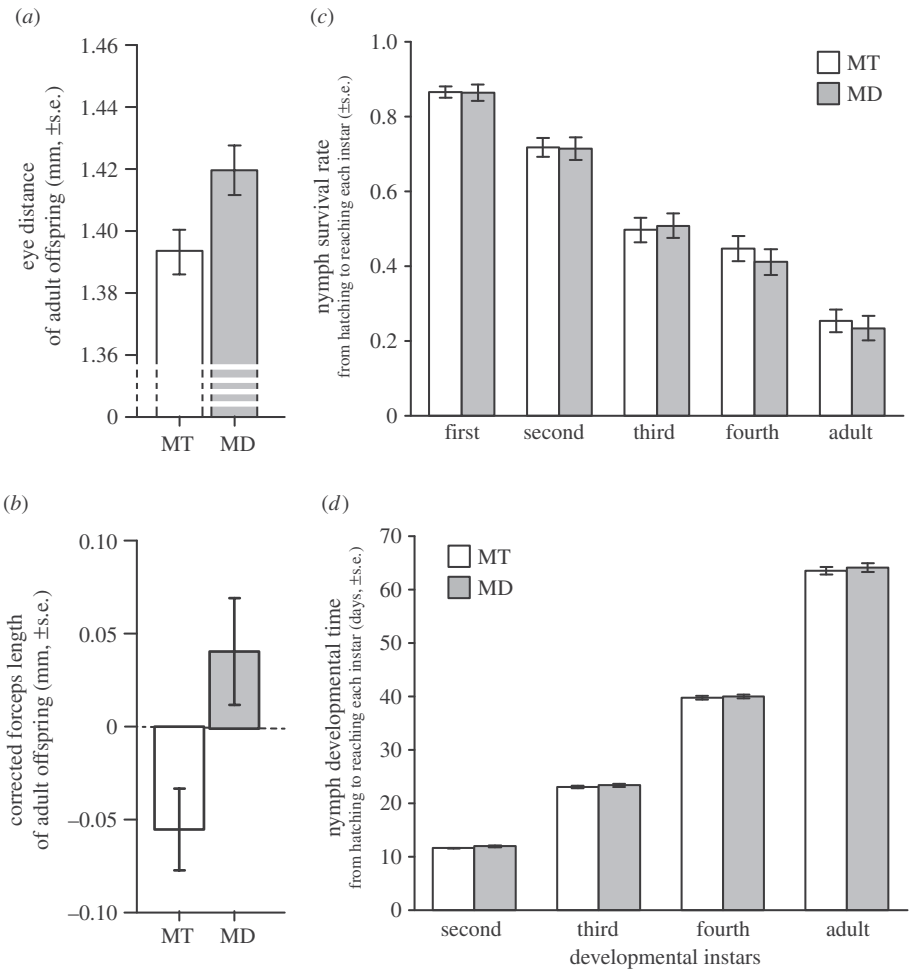

Figure 2. Short-term effects of maternal loss on the (*a*) mean eye distance, (*b*) corrected forceps length, (*c*) survival rate, and (*d*) developmental time of offspring. Nymphs were either maternally tended (MT) or maternally deprived (MD) during the 16 days following their emergence.

Table 1. Long-term and transgenerational effects of maternal loss on the expression of maternal care by adult offspring. (Maternal care was measured towards clutches of eggs or nymphs (clutch type), except for food provisioning, which was only expressed towards nymphs. Significant *p*-values are in bold. LR, likelihood ratio. Note that non-significant interactions are reported to allow comparison among models, but their removal did not qualitatively change the results.)

	(*a*) clutch defence		(*b*) clutch desertion time		(*c*) food provisioning	
	LR χ^2_1	*p*-value	LR χ^2_1	*p*-value	LR χ^2_1	*p*-value
clutch type (CT)	26.35	**<0.0001**	0.64	0.424	—	—
fMother	1.31	0.253	4.62	**0.032**	5.24	**0.022**
gMother	0.89	0.346	0.23	0.634	0.87	0.351
gFather	0.85	0.357	0.04	0.850	0.03	0.853
CT : gMother	3.95	**0.047**	0.09	0.758	—	—
gMother : gFather	0.46	0.498	0.62	0.431	4.78	**0.029**

with maternally-tended females (figure 3*c*; estim. ± s.e. = 1.25 ± 0.59; $t_{48} = 2.11$, $p = 0.041$), whereas the experimental background of the females had no effect on food provisioning when nymphs were sired by maternally-deprived males (estim. ± s.e. = −0.54 ± 0.60; $t_{48} = -0.90$, $p = 0.374$). Finally, maternally-deprived mothers produced nymphs, but not eggs, that were overall less defended by the caring foster mothers (figure 3*d*; Interaction in table 1*a*; nymph stage: estim. ± s.e. = −0.51 ± 0.25; $t_{67} = -2.04$, $p = 0.045$; egg stage: estim. ± s.e. = 0.12 ± 0.24; $t_{67} = 0.52$, $p = 0.606$). Note that all but two measurements of maternal care were independent of each other (electronic supplementary material,

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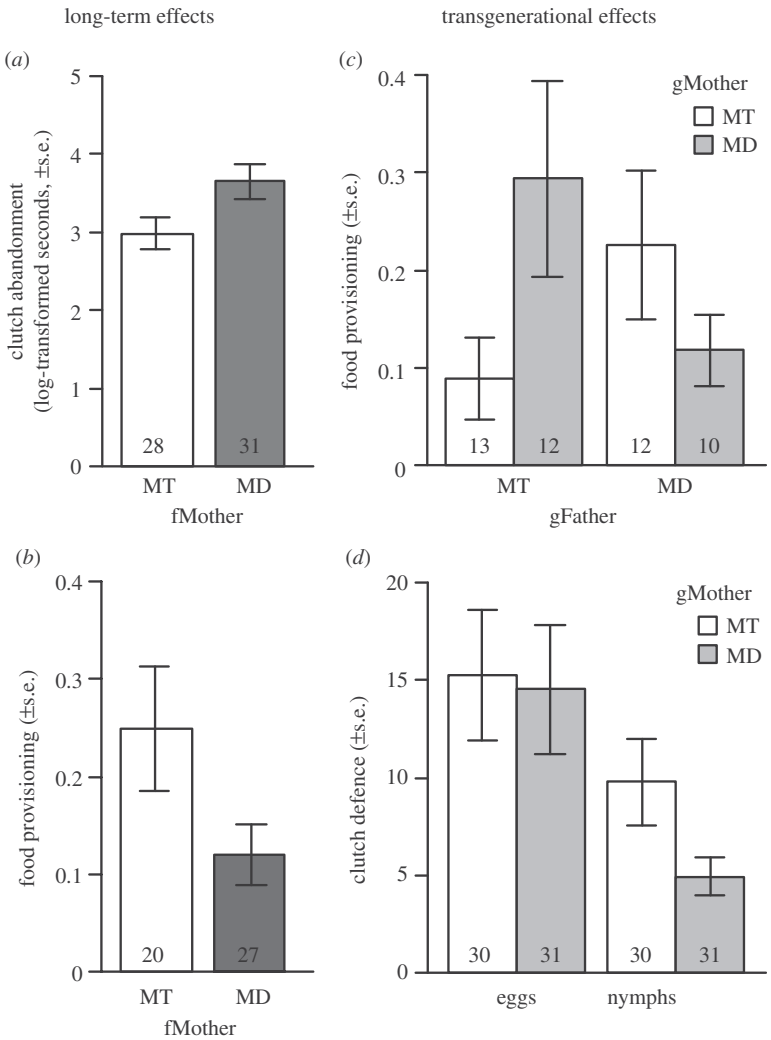

Figure 3. Long-term and transgenerational effects of maternal loss on the expression of (*a*) clutch abandonment, (*b,c*) food provisioning, and (*d*) clutch defence by adult offspring. MT, maternally tended; MD, maternally deprived. Sample sizes are at the bottom of each bar.

Table 2. Transgenerational effects of maternal loss on the reproductive output of the resulting adult offspring. (The significant *p*-value is in bold.)

	no. nymphs at egg hatching		mean weight of nymphs at egg hatching		developmental time of nymphs until adulthood		survival rate of nymphs until adulthood	
	LR χ^2_1	*p*-value	LR χ^2_1	*p*-value	LR χ^2_1	*p*-value	LR χ^2_1	*p*-value
gMother	1.43	0.232	1.02	0.312	6.19	**0.013**	1.04	0.307
gFather	0.33	0.568	0.08	0.776	0.73	0.394	0.33	0.564
fMother[a]	0.32	0.570	>0.01	0.991	—	—	—	—
maternal presence[a]	—	—	—	—	0.73	0.394	0.2	0.655

[a]Because fMother did not influence post-hatching traits measured in nymphs tended by a foster mother (electronic supplementary material, table S2), this factor was pooled to form a new factor describing the presence or the absence of mothers after hatching, then called 'maternal presence'.

table S3). Only egg defence and the duration of egg abandonment were overall positively correlated (Spearman correlation test, $r_s = 0.32$, $p = 0.009$).

Maternal loss influenced only one of the four measurements taken on nymphs and none of the three measurements taken on eggs produced by the adult offspring. Nymphs produced by maternally-tended females reached adulthood in 72.13 ± 0.67 days (mean $\pm$ s.e.), which was significantly longer than the 69.25 ± 0.51 days required by nymphs produced by maternally-deprived females (table 2). By contrast, the experimental background of the genetic parents had no effect on the number of eggs (gMother: LR $\chi^2_1 = 0.08$,

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$p = 0.782$; gFather: LR $\chi^2_1 = 0.06$, $p = 0.806$; interaction: LR $\chi^2_1 = 0.20$, $p = 0.657$), mean egg weight (gMother: LR $\chi^2_1 = 0.16$, $p = 0.688$; gFather: LR $\chi^2_1 = 0.92$, $p = 0.337$; interaction: LR $\chi^2_1 = 0.77$, $p = 0.380$) and hatching success (gMother: LR $\chi^2_1 = 0.01$, $p = 0.999$; gFather: LR $\chi^2_1 = 0.05$, $p = 0.827$; interaction: LR $\chi^2_1 = 2.07$, $p = 0.150$). The experimental background of the parents also had no effect on the number and mean weight of nymphs at egg hatching (table 2) or their survival until adulthood (table 2). Notably, the presence of a foster mother after egg hatching did not influence nymphs' survival rates and developmental times (table 2).

4. Discussion

Our experiments reveal the occurrence, but contrasting nature of short-term, long-term and transgenerational effects of maternal loss in the precocial insect *F. auricularia*. Under standard laboratory conditions, maternal loss entailed short-term benefits for adult offspring: maternally-deprived adults had wider eye distances and longer forceps than maternally-tended adults, two effects independent of nymph survival and/or developmental time. On the other hand, maternal loss entailed transgenerational costs. These costs were partly mediated by the experimental background of the caring mothers, as revealed by the longer clutch abandonment and lower food provisioning of maternally-deprived as compared to maternally-tended females. They were also partly mediated by the experimental background of the genetic parents: nymphs produced by maternally-deprived females were less well defended by their foster mother. Note, however, that when sired by maternally-tended males, nymphs produced by maternally-deprived females received more food than nymphs produced by maternally-tended females. Finally, we found a transgenerational effect of maternal loss on offspring developmental time: nymphs of maternally-deprived females reached adulthood earlier than nymphs of maternally-tended females. By contrast, there was no evidence for transgenerational effects of maternal loss on the other life-history traits measured in eggs and F_2 nymphs, irrespective of the presence or absence of a tending mother after egg hatching.

Somewhat surprisingly, we found that maternal loss did not affect nymph development and survival, but yielded adult offspring of larger body and forceps sizes, two morphological traits associated with fitness benefits in *F. auricularia* [22,23]. These apparent short-term benefits of maternal loss obtained under laboratory conditions contrast with the short-term costs typically expected under natural conditions (e.g. [4,28]). Previous studies already revealed that the maternal presence reduces nymph survival when families had restricted food access [21], but increased nymph survival when families had access to low quality food [15]. Here, the effects of maternal presence under ad libitum, high quality food could reveal an increased expression of sibling rivalry when juveniles have access to maternal resources, as proposed in a recent model [29]. Alternatively, these effects could result from maternal behaviours that benefit offspring under natural conditions, but directly or indirectly hamper their development under laboratory conditions. For instance, mothers often cover food in the vicinity of the nest, presumably to prevent microbial development (J. Kramer & J. Meunier 2015, personal observation). This might have been costly for juveniles in the absence of pathogens, as it might have restricted their access to food. This notwithstanding, both scenarios emphasize that the parental presence can be associated with costs for the tended offspring (see also [21]), which emerge when the (laboratory) conditions do not allow (variation in) the benefits of parental care to be revealed. These findings overall indicate that as long as parental care only has limited effects on offspring development and survival (a scenario that presumably prevailed in the early evolution of family life), it is likely that the emergence and maintenance of parental investment into post-hatching care mostly relies on the benefits of parenting behaviours that enable offspring to better cope with environmental constraints, such as limited food access or the presence of pathogens and predators [2,3].

Contrary to the above effects, maternal loss lowered the expression of maternal care by adult offspring. Females reared without mothers abandoned their juveniles for longer and provisioned them with less food than females reared with tending mothers. To the best of our knowledge, the long-term, negative effects of maternal loss on the expression of maternal care have only been reported in altricial vertebrates [8–10,13]. In these species, the effects of maternal loss typically result from a disrupted learning process [30] and/or from induced hormonal/neurobiological changes during juvenile development. For instance, temporary maternal deprivation is known to alter the brain development of juvenile rodents and primates, which in turns disturbs hormonal and neurobiological processes in adults and then hampers their expression of parental care (e.g. [31,32]). In altricial insects like honeybees and dung beetles, the absence of brood care is also known to delay the development of sensory and integrative brain centres in juveniles (e.g. antennal lobes and mushroom bodies [33,34]). However, the link between these developmental changes and the expression of care remains unknown. Our results thus call for further studies that test the effects of maternal loss on hormonal/neurobiological traits in juveniles of (precocial) invertebrates. Moreover, they suggest that effects of learning [35] on the expression of parenting behaviours might not be restricted to vertebrates. Finally, the occurrence of negative effects of parental loss in a precocial insect demonstrates that their expression is not restricted to species in which parental care is obligatory for offspring survival (such as altricial vertebrates). Note that the larger body and forceps size of maternally-deprived adults are unlikely to be the main drivers of these long-term negative effects on maternal care, as a previous study showed that large *F. auricularia* females express more rather than less maternal care (including food provisioning) than small females [17].

Importantly, the effects of maternal loss on the expression of maternal care were not only mediated by parental effects reflecting the experimental background of the caring mothers, but also by effects of the background of the parents that produced the nymphs. On the one hand, nymphs of maternally-tended females moulted into adults at a greater age than nymphs of maternally-deprived females. On the other hand, nymphs produced by maternally-deprived females were less well defended by foster mothers, but—when fertilized by maternally-tended males—received more food from foster mothers than nymphs produced by maternally-tended females. These results are overall in line with studies demonstrating that parent-of-origin effects inherited to and expressed by juveniles are key components of

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parental care [20,24,25,36]. Interestingly, our findings also reveal that these effects can be acquired from the early social environment of the future parents (e.g. through epigenetic modifications [37]), showing the central importance of this phenomenon in the evolution of parental investment into care in a precocial insect. Under our experimental conditions, these effects did not translate into the production of high quality nymphs by maternally-deprived females, as we found no parent-of-origin effect of maternal loss on the mean weight and survival rate of these nymphs. However, the accelerated developmental time of the offspring of maternally-deprived females could be indicative of parent-of-origin effects that increase the level of sibling rivalry among these offspring [15,21]. On a proximate level, the transgenerational effects of maternal loss on nymph defence could be mediated by a parent-of-origin effect on the offspring's chemical signatures. These signatures are known to mediate behavioural interactions between earwig mothers and nymphs as well as among nymphs, to be flexible over time, and to determine the amount of food provisioned by mothers [38].

To conclude, our study demonstrates that maternal loss during earwig family life may entail short-term benefits regarding adult morphology, but is associated with transgenerational costs that are mainly mediated through the expression of parental care. These contrasting effects stress the importance of encompassing the short- and long-term effects of the parental presence when estimating the costs/benefits ratio of mother–offspring interactions for offspring. More generally, the surprising short-term benefits of maternal loss obtained under laboratory conditions shed light on the importance of (natural) environmental constraints on the net benefits gained by juveniles through parental care and on their role in the evolution of family life [3]. Finally, our data reveal that the early social environment of juveniles is a key determinant of parental investment across generations, as it shapes both the behaviour they later adopt as parents and the behaviours they transmit to their own offspring. Hence, this study overall suggests that environmental constraints and transgenerational effects of parental loss are keystones in the emergence and maintenance of ancestral forms of family life, in which parental care has limited effects on offspring development and survival [16].

Data accessibility. Data are deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.sm6n2.

Authors' contributions. J.T., J.K., L.K.K. and J.M. conceived the experiment. J.T., J.K. and L.K.K. performed the experiment. J.M. analysed the data. J.M. and J.K. wrote the first draft of the manuscript. All authors approved the final manuscript.

Competing interests. We declare we have no competing interests.

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Inbreeding depression in an insect with maternal care: influences of family interactions, life stage and offspring sex

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sibling rivalry;
sib mating.

Abstract

Although inbreeding is commonly known to depress individual fitness, the severity of inbreeding depression varies considerably across species. Among the factors contributing to this variation, family interactions, life stage and sex of offspring have been proposed, but their joint influence on inbreeding depression remains poorly understood. Here, we demonstrate that these three factors jointly shape inbreeding depression in the European earwig, *Forficula auricularia*. Using a series of cross-breeding, split-clutch and brood size manipulation experiments conducted over two generations, we first showed that sib mating (leading to inbred offspring) did not influence the reproductive success of earwig parents. Second, the presence of tending mothers and the strength of sibling competition (i.e. brood size) did not influence the expression of inbreeding depression in the inbred offspring. By contrast, our results revealed that inbreeding dramatically depressed the reproductive success of inbred adult male offspring, but only had little effect on the reproductive success of inbred adult female offspring. Overall, this study demonstrates limited effects of family interactions on inbreeding depression in this species and emphasizes the importance of disentangling effects of sib mating early and late during development to better understand the evolution of mating systems and population dynamics.

Introduction

Inbreeding is considered as one of the key parameters in the persistence of natural populations, as well as in the evolution of social systems, life history, morphology, physiology and behaviour (Keller & Waller, 2002; Frankham, 2008; Enders & Nunney, 2010). Inbreeding results from the mating of two related individuals such as brothers and sisters (i.e. sib mating). Sib mating is known to increase the degree of homozygosity in the offspring, which in turn can depress offspring fitness through the higher expression of deleterious recessive alleles or the induced reduction in heterozygote advantages (Charlesworth & Charlesworth, 1987; Roff, 2002). The negative impact of inbreeding on fitness-related traits, called inbreeding depression, has been shown to vary considerably across taxa, species and even populations (Crnokrak & Roff, 1999; Keller & Waller, 2002). For instance, some studies reported that sib mating dramatically reduces fitness-related traits such as the production and the survival of offspring (e.g. Keller, 1998; Slate *et al.*, 2000; Saccheri *et al.*, 2005; Vitikainen *et al.*, 2011). Conversely, other studies found that inbreeding had limited effects on fitness-related traits (e.g. Kureck *et al.*, 2012; Mehlis *et al.*, 2012) or may even provide fitness benefits for the two mating partners (e.g. Waser *et al.*, 1986; Lehmann & Perrin, 2003; Cohen & Dearborn, 2004; Neff, 2004; Kokko & Ots, 2006; Thünken *et al.*, 2007; Tabadkani *et al.*, 2012).

Across animal and plant species, multiple sources of environmental stress have been shown to exacerbate the negative effects of inbreeding, such as exposure to parasites or chemicals, food deprivation, competition and increase in the density of individuals in a population or a group (Armbruster & Reed, 2005; Konior *et al.*, 2005; Fritzsche *et al.*, 2006; Zajitschek *et al.*, 2009; Fox & Reed, 2010; Michalczyk *et al.*, 2010; Reed *et al.*,

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2012). The general importance of environmental stress on inbreeding depression recently received quantitative support from a meta-analysis based on 33 plant and animal species. In this study, Fox & Reed (2010) showed a positive and linear relationship between the severity of environmental stresses (listed above) and the lethal effects of inbreeding depression. In this analysis, environmental stresses explained 41% of the variance in the lethal effects of inbreeding depression measured across studies, which also indicates that other factors are (at least partly) responsible for the observed variation in the expression and magnitude of inbreeding depression (Keller & Waller, 2002; Armbruster & Reed, 2005; Waller *et al.*, 2008).

Family life is a widespread phenomenon in nature that is often associated with competition between siblings over resources and parental care (Smiseth *et al.*, 2012; Wong *et al.*, 2013), two forms of social interactions that possibly shape the severity of inbreeding depression. Sibling rivalry is an important source of social stress that is known to negatively affect the development and/or the survival of offspring (reviewed in Roulin & Dreiss, 2012) and is typically intensified in large family groups and under limited food resources (reviewed in Mock & Parker, 1997). Conversely, the presence of parents is commonly associated with benefits to offspring, for instance through the provisioning of resources necessary for offspring development and survival (Smiseth *et al.*, 2012). Hence, intense sibling rivalry is predicted to exacerbate inbreeding depression, and parental care expected to (at least partly) impede detrimental inbreeding depression (i.e. buffer against the poor quality of inbred offspring; Avilés & Bukowski, 2006). To date, the effects of sibling competition (during family life) on inbreeding depression remain largely untested, whereas mixed conclusion emerged from the few studies that examined the influence of maternal care on inbreeding depression. In the prairie voles, *Microtus ochrogaster*, inbreeding status of the tended offspring has been shown to not influence the level of parental care (Bixler & Tang-martinez, 2006). Conversely, the absence of inbreeding depression in offspring of the oldfield mice *Peromyscus polionotus*, the cichlid fish *Pelvicachromis taeniatus* and the subsocial spider *Anelosimus jucundus* has been suggested to result from the occurrence of parental care in these species (Margulis, 1997, 1998; Avilés & Bukowski, 2006; Thünken *et al.*, 2007), although such a causal link remains to be tested.

Whether or not parents should provide more care towards inbred offspring (and thus counter inbreeding depression) may also depend on the cost of care. For instance under resource limitation, parents may weigh their own survival and/or future reproduction higher than the fitness of their current offspring and consequently compete with offspring for the limited resources, which may in turn exacerbates inbreeding depression. Mother–offspring competition over limited food resources has been recently described in the European earwigs, *Forficula auricularia*. When earwig families were reared under restricted food conditions, offspring suffered from maternal presence in terms of slower development and lower survival rates (Meunier & Kölliker, 2012b). Nevertheless, even under restricted food conditions, earwig offspring continue to actively aggregate with their mothers, possibly because other forms of care (e.g. protection against predators) continue to outweigh the cost of mother–offspring competition over food (Wong & Kölliker, 2012). Hence, under restricted food availability, the presence of mothers possibly becomes an environmental (i.e. nutritional) stressor that may exacerbate the effects of inbreeding depression in offspring.

The severity of inbreeding depression may also vary depending on the life stage of the inbred individuals (i.e. the progeny ensuing from sib mating). In populations with some inbreeding history, inbreeding depression is predicted to purge the highly deleterious recessive alleles, which typically affect early stages (such as embryonic development) and thus entail lower levels of inbreeding depression in early than late life stages (Husband & Schemske, 1996). Conversely, in populations without inbreeding history, the maintenance of these deleterious recessive alleles may dramatically affect the early life stages of individuals in case of inbreeding and thus result in higher levels of inbreeding depression in early than late life stages (Husband & Schemske, 1996). These predictions received support from a literature survey conducted in plants, where the predominantly outbreeding species showed greater inbreeding depression in early stages than the predominantly selfing species (Husband & Schemske, 1996). In a population of the subsocial spider, *A. cf jucundus*, where inbreeding is likely to occur, inbreeding depression was also detected in the late but not the early part of the life cycle (Avilés & Bukowski, 2006). Interestingly in this spider species, females provide care to their offspring only during early life stages, which could also explain the absence of inbreeding depression specifically at this stage (Avilés & Bukowski, 2006).

Finally, male and female offspring may exhibit different sensitivity to inbreeding depression. Inbreeding depression has been shown to specifically affect several fitness-related traits in males, such as fertilization success (Pray *et al.*, 1994; Saccheri *et al.*, 2005; Enders & Nunney, 2010), territory acquisition success (Potts *et al.*, 1994; Meagher *et al.*, 2000) or success in sperm competition (Konior *et al.*, 2005; Fritzsche *et al.*, 2006; Zajitschek *et al.*, 2009; Michalczyk *et al.*, 2010). Conversely, female-specific effects of inbreeding depression have been demonstrated in humans, where pedigree data from a Swiss mountain village population revealed that the most inbred mothers (but not fathers) had significantly fewer children (Postma *et al.*, 2010).

Although the sex-specific effect of inbreeding on individual reproductive success has been described in several species, it remains unclear to what extent mating with an unrelated but inbred partner alters the fitness of noninbred individuals. In particular, inbred males have been shown to either substantially reduce (Fox *et al.*, 2011; Okada *et al.*, 2011) or to not shape (Michalczyk *et al.*, 2010) the fitness of the noninbred and unrelated females they mated with, whereas inbred females had limited effects on the fitness of the noninbred and unrelated males they mated with (Enders & Nunney, 2010).

The aim of this study was to disentangle whether inbreeding depression in the European earwig, *Forficula auricularia*, is shaped by mother–offspring and sibling competition over restricted food access, by life stages (early vs. late) and by the sex of adult offspring. In this promiscuous insect species, females tend their clutch of eggs and nymphs for several weeks and provide care in the forms of protection against natural enemies and food provisioning to nymphs (reviewed in Costa, 2006). Food restriction and large family groups (i.e. large clutches) are known to be major sources of stress that reduces the survival of earwig offspring and shape population density (Kölliker, 2007; Moerkens *et al.*, 2009). The presence of mothers has been shown to enhance offspring survival under natural and good food conditions in the laboratory (Kölliker, 2007; Kölliker & Vancassel, 2007). Conversely, these benefits of maternal care are reduced under food restriction, possibly due to mother–offspring competition over the scarce food (Meunier & Kölliker, 2012b). Here, we used a series of experiments conducted under environmentally stressful conditions (limited food availability) to test whether (i) sib mating decreases the reproductive success of females due to the production of inbred offspring, (ii) sibling competition and/or mother–offspring competition over limited food resources exacerbate the effects of inbreeding depression in offspring, and finally whether (iii) inbreeding depression in the resulting inbred adult offspring is sex-specific. The results of these experiments will also reveal whether inbreeding depression is more (or less) severe in later than early life stages of individuals (i.e. juvenile development/survival and adult reproductive success) and to what extent mating with an inbred (but unrelated) male or female affects the fitness of the noninbred mating partner.

Materials and methods

Origin of the tested individuals

The males and females used in the following experiments were the first laboratory-born generation from a field-sampled population of *F. auricularia* collected in May 2009 in Dolcedo, Italy. The sampling was made using 50 wood traps regularly spread over a 3000 m^2 area to limit the risk of high relatedness among the collected individuals. The resulting field-sampled population was composed of 600 males and 600 females sampled as (virgin) fourth instar nymphs and maintained under standardized laboratory conditions for the rest of their life cycle. These standardized laboratory conditions are detailed in Meunier *et al.* (2012). Briefly, groups of 30 newly emerged males and 30 newly emerged females were maintained in large plastic containers for 5 months to allow mating (this species has a promiscuous mating system). They were kept at 60% humidity, at 14:10-h light–dark photoperiod and at a constant temperature of 20 °C (later called laboratory summer conditions) and provided *ad libitum* food [see food composition in Meunier *et al.* (2012)]. The resulting mated females were then isolated in Petri dishes (diameter 10 cm), in which they later produced their first clutch of eggs (approx. 50 eggs per female, see Meunier *et al.*, 2012). From egg laying to egg hatching, females were maintained under complete darkness at 15 °C and 60% humidity (later called winter conditions), and no food was provided during this period (Kölliker, 2007). Eggs hatched approx. 24 days later (see Meunier *et al.*, 2012). On day one after hatching of a clutch, the family (mothers and offspring) was transferred to a new Petri dish and received *ad libitum* food (see Meunier *et al.*, 2012). The females were separated from their nymphs on day 14 after hatching, which corresponds to the approximate termination of food provisioning in *F. auricularia* (Wong & Kölliker, 2012). The mothers were isolated in new Petri dishes with *ad libitum* food until day 60 after hatching of their first clutch, during which 82% of them produced a second clutch (see Meunier *et al.*, 2012). Simultaneously, 20 nymphs per clutch were setup in new large Petri dishes (diameter 14 cm) until they reached adulthood. The newly emerged adults were immediately separated by sex to avoid uncontrolled sib mating. These adult offspring were the individuals used in the present study.

Part 1: Effects of sib mating on parental reproductive success

Adult offspring produced by 46 females randomly chosen from the field-sampled population were used to test the effect of sib mating on offspring development and survival. Among them, 23 were experimentally mated to a genetically unrelated male (control), and 23 to one of their brothers (sib mating, Fig. 1a). These experimental matings were conducted by placing each virgin female with one virgin male (always different) in Petri dishes (diameter 10 cm) containing humid sand, a plastic shelter used as a nest and *ad libitum* food. After 4 weeks, males were removed from the Petri dishes and the females subsequently placed under laboratory winter conditions until egg hatching. At hatching, a random sample of 10 nymphs per clutch was weighed

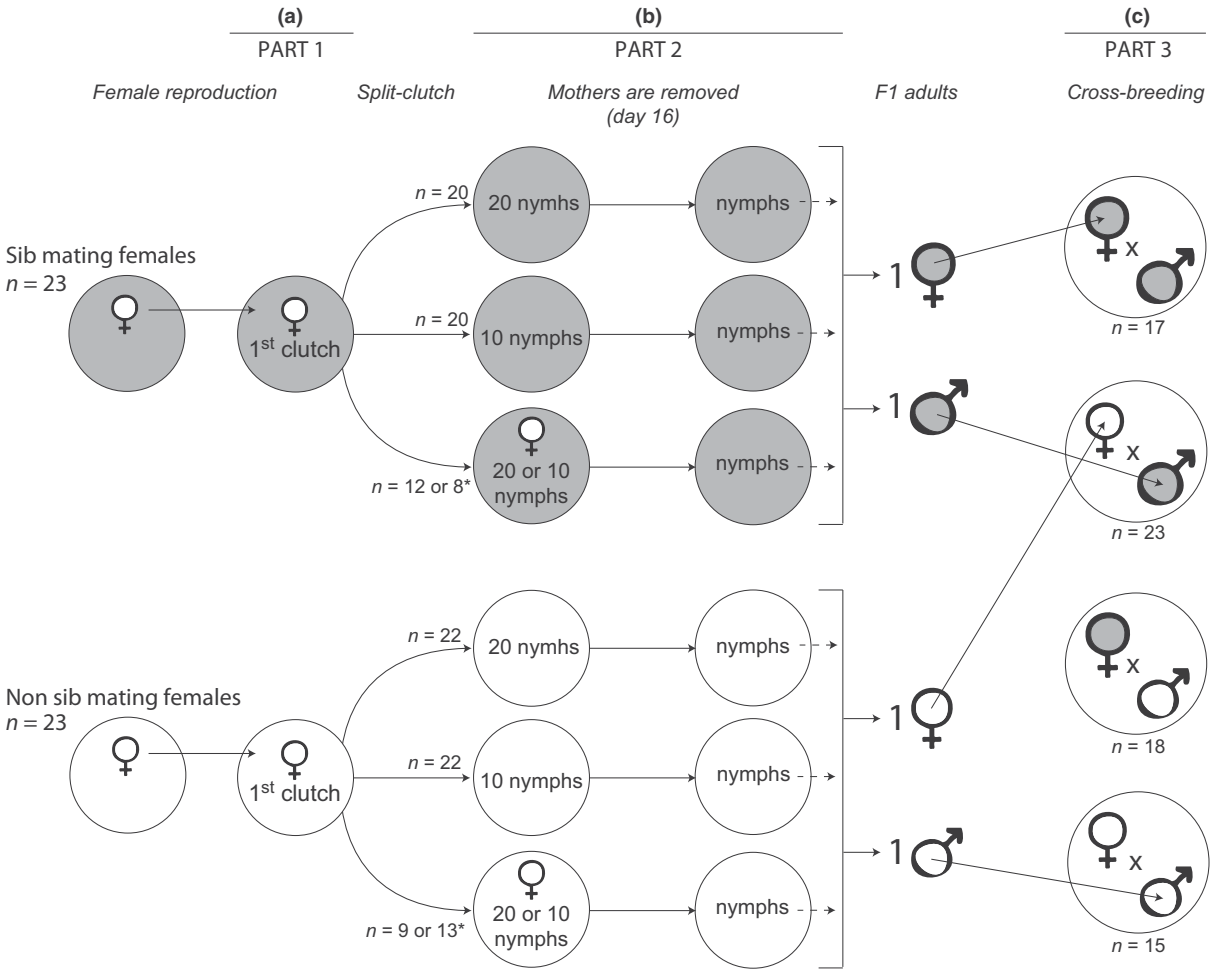

Fig. 1 Details of the experimental set-up allowing to (a) test the effects of sib mating of parental reproductive success, (b) disentangle the effects of offspring inbreeding status and social environment on nymphs development and survival rates and finally to (c) investigate the effects of adult inbreeding status on their reproductive success. *respectively.

to the nearest 0.001 mg using a Mettler-Toledo MT5 Micro-balance (Mettler, Roche, Basel) to estimate the quality of newly produced nymphs. Then, the females and their clutches were transferred to new Petri dishes (according to the experimental design detailed in the Part 2; see also Fig. 1), in which they were kept under laboratory summer condition for 16 days. Females were then isolated in new Petri dishes to allow second clutch production.

The influence of sib mating on the reproductive success of females was estimated in both first and second clutches using the four following measurements. First, we counted the number of eggs produced by each female 3 days after the first egg has been observed. In this species, all eggs are typically released within 1 day, but a very limited number of females may still produce a few eggs during the 2 days following the first egg release (J. Meunier & M. Kölliker, unpublished data). Second, we counted and weighed newly hatched nymphs the day after the first nymph has been observed. Egg hatching is particularly well synchronized in this species, where 97 ± 1% (mean ± SE, n = 46) of the newly produced nymphs emerge over the first day (Meunier & Kölliker, unpublished data). Third, we used the number of eggs produced and the number of hatched nymphs to calculate the hatching success of each clutch. Finally, we tested whether sib mating influenced the relative investment of females in their second clutches (compared with their first clutch), because this trait is known to vary naturally between females, to partly reflect the outcome of family conflicts and to be associated with other important life-history traits, such as the lifetime number of eggs produced or the level of food mothers provision to their offspring

(Meunier & Kölliker, 2012a; Meunier *et al.*, 2012). This relative investment was calculated by dividing the number of eggs produced in the second clutches by the lifetime number of produced eggs.

Part 2: Effects of inbreeding status on offspring development and survival

The effects of sib mating on the development and survival of offspring under high levels of environmental (social) stress were then tested using a split-clutch experimental design in which we manipulated both brood size and maternal presence (Fig. 1b). One day after hatching, 42 of the 46 above clutches (in the following referred to as 'clutch of origin') were divided into three experimental groups (notice that four clutches – three inbred and one noninbred – were excluded because they produced fewer than 40 nymphs): one group with a brood size of 10 nymphs (small group) and without mother, one group with a brood size of 20 nymphs (large group) without mother and one group with the mother and either 10 or 20 of its nymphs (Fig. 1b). This experimental design allowed us to generate a two-by-two complete block design with the presence/absence of mother and large/small brood size for both sib mating and control families. The 126 resulting experimental broods were maintained in small Petri dishes (diameter 6 cm) for the next 16 days. At that time, we counted the proportion of surviving nymphs that reached the second juvenile instar as a measure of developmental rate. All nymphs were subsequently transferred to new Petri dishes for the next 15 days, and then to large Petri dishes (diameter 14 cm) until they reach adulthood. The mothers were then isolated to allow second clutch production (see Part 1). Upon emergence as adults, we (i) determined the sex of all individuals, (ii) checked for the occurrence of developmental problems in terms of, for instance, anatomical 'hermaphroditism' (i.e. showing one female-like and one male-like forceps, a phenomenon sometimes also associated with mixed internal anatomy; Günther & Herter, 1974), wing cover abnormalities, mis-shaped forceps or antennae, (iii) counted the number of adults for each family for analyses of survival rates and (iv) separated brothers and sisters from each experimental group to prevent uncontrolled sib mating. The individual weight of adult male and female offspring was measured to the nearest 0.001 mg about 1 month after emergence and then averaged per family group. Because the weight of adults with developmental problems was generally much smaller than the one of adults from the same clutch with normal development, the former were excluded from the calculations of adult mean weights (note that their inclusion did not qualitatively change the results).

Because restricted food conditions are known to create a stressful environment that exacerbates the negative effects of both sibling competition and mother–offspring competition over food resources in earwigs (Meunier & Kölliker, 2012b), the experimental earwig broods received a limited amount of the food. Specifically, food was provided every 6 days, and any leftover food was removed 3 days after supply (Meunier & Kölliker, 2012b). The quantity of food was adjusted according to the age of the nymphs, with 60, 120 and 240 mg of food provided in the small, normal and large Petri dishes, respectively. Females had access to *ad libitum* food once isolated for second clutch production. From hatching until adulthood, clutches were kept under laboratory summer conditions.

Part 3: Sex-specific effects of adult inbreeding status on reproductive success

We finally used a simple cross-breeding design involving adults produced in the 126 previously detailed experimental first clutches to disentangle the effects of inbreeding on adult males and females on their reproductive success (Fig. 1c). To this end, we haphazardly sampled one male and/or one female per clutch of origin across the three types of experimental clutches. These adults were then paired in new Petri dishes to obtain the four possible mating combinations: inbred female + inbred male, inbred female + outbred male, outbred female + inbred male and outbred female + outbred male. Each pair contained individuals from different families to avoid a second generation of inbreeding, and each combination had the same proportion of individuals previously reared in each type of experimental clutch. The sample size of each combination varied due to the number of males and females available in each experimental clutch. Three inbred families provided two males in the experiment.

The experimental mating and the rearing conditions until egg hatching were similar to the ones described above. At hatching, females were transferred with their nymphs in new Petri dishes for 16 days under laboratory summer conditions. At day 16, the number of nymphs was counted and the nymphs were discarded. The females were isolated in new Petri dishes to quantify second clutch production. When the first clutch eggs did not hatch, females were isolated in new Petri dishes 36 days after egg laying (a duration that approx. corresponds to the mean 23 days between oviposition and hatching of eggs, and 16 days of post-hatching family life, see above and Meunier *et al.*, 2012). The sex-specific effects of adult inbreeding status were then tested by quantifying (in both first and second clutches) the number of eggs produced, the hatching success, the number of nymphs and the relative investment in second clutches (see above) of the mated females. The mean weight of newly hatched nymphs was also measured in the first clutches.

Statistical analyses

We first compared the reproductive success of females that were mated to their brother to the one of females that were mated to an unrelated male using a multivariate analyses of variance (MANOVA). In this analysis, the measured traits (i.e. egg numbers in 1st and 2nd clutches, hatching success in 1st and 2nd clutches, nymph number and weight in 1st and 2nd clutches, and finally relative investment in second clutches) were entered as dependent variables and the mating type (sib mating vs. control) as fixed factor. Because the likelihood of second clutch production and the lifetime production of eggs and nymphs were tightly correlated with the number of eggs and nymphs produced in each or both clutches, these three measurements were not entered in the MANOVA but analysed separately (see Results). To confirm that traits of the second clutches were not shaped by the fact that females experimentally tended either 10 or 20 of their first clutch nymphs, we conducted another MANOVA where the traits of the second clutch (i.e. egg numbers in 2nd clutches, hatching success in 2nd clutches, nymph number and weight in 2nd clutches and relative investment in second clutches) were entered as dependent variables, and the mating type (sib mating vs. control), the tended brood size (small or large) and their interaction as fixed factors.

We then investigated the effects of maternal presence and increased sibling competition on inbreeding depression measured both in nymphs (nymph development rate, survival rate of nymphs until adulthood) and in adults (absolute number of adults per family, clutch sex ratio, mean weight of males and females, proportion of adults with developmental problems). To this end, we used generalized linear mixed models (GLMMs) with Gaussian or when required, binomial error distribution, where mating type (sib mating vs. control), maternal presence and experimental brood size were entered as fixed factors, and the clutch of origin as random factor. The GLMM on clutch sex ratio and mean weight of males and females also included the total number of adults per family group as covariate. All interactions were tested and removed from the GLMMs when non-significant (all $P > 0.12$). Interactions between inbreeding and brood size and between inbreeding and maternal presence were kept for interpretation, but the results do not qualitatively change if they are removed from the models.

Finally, we analysed the influence of sib mating on the reproductive success of male and female offspring using a series of randomized general linear models (randomized GLMs; Manly, 1997). This method was used because the overall hatching success from the cross-breeding experiment was relatively low (mean ± SD: 16.3 ± 31.6%) so that the distribution of model residuals could not be properly tested. Randomized GLM consisted in using the type of father (inbred or outbred), the type of mother (inbred or outbred) and their interaction as fixed factors, although each of the 11 measures reflecting parental reproductive success (the 11 response variables are listed in Table 4) was randomly permuted 10 000 times within factors (R script available on demand, see Meunier *et al.*, 2008). All statistical analyses were conducted using R v2.15.1.

Results

Part 1: Effects of sib mating on parental reproductive success

Overall, the reproductive success of females mated to a brother was not significantly different from the one of females mated to an unrelated male (Table 1, MANOVA conducted on the number of eggs produced in the 1st and 2nd clutches, their hatching success, the number and weight of nymphs produced in each of these clutches and the relative investment of females into their second clutch; *Wilk's Lambda* = 0.64, approx. $F_{9,27} = 1.67$, $P = 0.15$). Sib mating had also no significant effect on the likelihood of 2nd clutch production (Table 1; Fisher's exact test, $P = 1.00$), the lifetime production of eggs (*t*-test, $t_{44} = -0.24$, $P = 0.82$) and the lifetime number of hatched nymphs (*t*-test, $t_{44} = -1.41$, $P = 0.16$). Furthermore, measures of second clutches were not significantly influenced by our manipulation of first brood size (MANOVA; *Wilk's Lambda* = 0.89, approx. $F_{5,28} = 0.67$, $P = 0.65$), sib mating

Table 1 Short-term fitness correlates of females mated to an unrelated male (control) or to a brother (sib mating).

	Control		Sib mating	
	Mean ± SE	*N*	Mean ± SE	*N*
First clutch				
Egg number	66.52 ± 2.23	23	62.70 ± 1.69	23
Hatching success (%)*	83.95 ± 3.97	23	73.15 ± 5.71	23
Newly hatched nymphs	55.35 ± 3.01	23	45.09 ± 3.46	23
Mean nymph weight (mg)	1.45 ± 0.03	23	1.49 ± 0.05	23
Second clutch				
Egg number	34.48 ± 4.05	23	36.70 ± 3.86	23
Hatching success (%)*	79.35 ± 6.83	19	74.99 ± 4.84	20
Newly hatched nymphs	28.09 ± 3.96	23	27.30 ± 3.30	23
Mean nymph weight (mg)	1.46 ± 0.03	17†	1.46 ± 0.05	20
General				
Likelihood of 2nd clutch prod. (%)	82.61	23	86.96	23
Relative investment in 2nd clutch	31.16 ± 3.43	23	33.90 ± 3.20	23
Lifetime egg number	101.00 ± 4.87	23	99.39 ± 4.81	23
Lifetime newly hatched nymph	83.43 ± 5.47	23	72.39 ± 5.58	23

*Hatching success was calculated using egg-laying females only.
†Two clutches did not produced any nymph.

(*Wilk's Lambda* = 0.74, approx. $F_{5,28}$ = 1.96, P = 0.12) or an interaction between these two factors (*Wilk's Lambda* = 0.82, approx. $F_{5,28}$ = 1.26, P = 0.31).

Part 2: Effects of inbreeding status on offspring development and survival

The effect of sib mating on nymph developmental rate depended on maternal presence, as revealed by the significant interaction term between sib-/nonsib mating and presence/absence of mothers (Table 2). In the presence of mother, no significant inbreeding depression was observed (Welch's *t*-test, t_{38} = 0.71, P = 0.484), whereas in the absence of mothers, the developmental rate of inbred nymphs was reduced by 51% relative to outbred nymphs (Fig. 2, Welch's t-test, t_{39} = −2.16, P = 0.037). Conversely, sib mating had no significant effect (neither as main effect, nor in an interaction with maternal presence and experimental brood size) on the survival rate of nymphs until adulthood, the absolute number of adult produced, the sex ratio of adults produced or the mean body weight of newly produced males and females (Tables 2 and 3).

The proportion of offspring turning into adults with developmental problems was significantly smaller in the presence of a tending mother (1.7% of 116 adults) than in the absence of a mother (5.7% of 418 adults, Binomial GLMM, LR χ^2_1 = 6.27, P = 0.012). This proportion was slightly smaller in outbred (3.4% of 295 adults) than inbred groups (6.7% of 239 adults), albeit this difference was not significant (Binomial GLMM, LR χ^2_1 = 3.41, P = 0.065). Neither brood size (Binomial GLMM, LR χ^2_1 = 1.62, P = 0.20), interactions between maternal presence and inbreeding (Binomial GLMM, LR χ^2_1 = 1.44, P = 0.23) or between brood size and inbreeding (Binomial GLMM, LR χ^2_1 = 0.69, P = 0.41) significantly influenced the proportion of adults with developmental problems.

Independently from mating type, nymph developmental rate and survival until adulthood were significantly higher in small as compared with large experimental clutches (Table 2, Developmental rate in small clutches: mean ± SE = 0.31 ± 0.04 and in large clutches: 0.15 ± 0.04; survival rate in small clutches: 0.42 ± 0.03 and large clutches: 0.20 ± 0.01). As predicted under restricted food availability where females may compete with offspring over the scarce food (see Introduction), the clutches reared in the absence of mothers overall exhibited a higher nymph survival rate until adulthood and produced a larger absolute number of adults than the clutches reared in the presence of a mother (Table 2; survival rate: absence = 0.36 ± 0.02, presence = 0.22 ± 0.03; absolute number of adults: absence = 4.69 ± 0.21, presence = 2.71 ± 0.38). Our results also show that brood size and maternal presence had no significant effect on the adult sex ratio of clutches and the mean weight of newly produced males and females (Table 3). Finally, the total number of adult offspring produced in a clutch significantly decreased the weight of newly produced females, but had no significant effect on the weight of newly produced males (Table 3, Fig. S1).

Part 3: Sex-specific effects of adult inbreeding status on reproductive success

Sib mating of parents significantly reduced the reproductive success of the (correspondingly inbred) male offspring, but had only limited negative effects on the reproductive success of the female offspring. Of the 10 measures taken to characterize the reproductive success of inbred males and females, four were significantly influenced by the inbreeding status of the father, one by the inbreeding status of the mother and none by an interaction between the inbreeding status of the two parents (Tables 4 and S1). In particular, comparing our measures of reproductive success between inbred and outbred males, the hatching success of first clutch eggs was 75% lower, the number of first clutch nymphs 70% lower, the number of eggs in the second clutches 35% lower and the lifetime number of nymphs 64% lower.(Fig. 3, Table 4). By contrast, inbred and noninbred females showed only marginally significant differences in measures of reproductive success, with the

Table 2 Influence of inbreeding status (inbred vs. noninbred) and two sources of stress (maternal presence and brood size) on nymph development and adult production. Statistics obtained using GLMM.

	Nymph developmental rate		Nymph survival rate until adulthood		Absolute number of adults produced	
	LR χ^2_1	*P*-value	LR χ^2_1	*P*-value	LR χ^2_1	*P*-value
Inbreeding status	3.34	0.068	1.44	0.230	1.75	0.186
Maternal presence	46.89	**<0.0001**	24.77	**<0.0001**	33.46	**<0.0001**
Experimental brood size	63.04	**<0.0001**	71.00	**<0.0001**	0.12	0.728
Inbreed. stat.: Maternal presence	6.38	**0.012**	0.01	0.916	<0.01	0.974
Inbreed. stat.: Exp. brood size	0.84	0.357	0.13	0.577	0.13	0.723

*Significant *P*-values are in bold. LR, likelihood ratio.

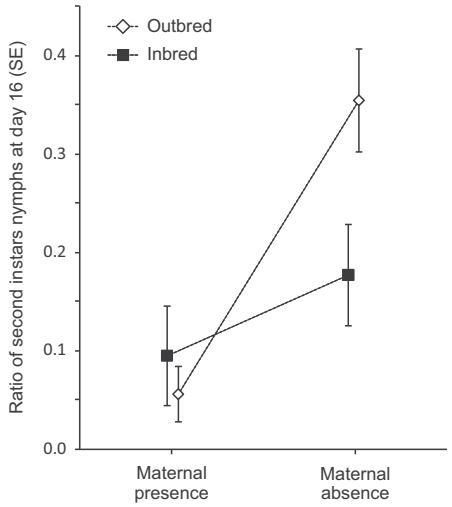

Fig. 2 Interacting effect of inbreeding and maternal presence on nymph developmental rate.

inbred females having first clutch nymphs 12% heavier than outbred females, and inbred females producing 28% fewer second clutch eggs than outbred females (Fig. 3; Tables 4 and S1).

Discussion

This study demonstrates that offspring life stage and sex had strong influence on the expression of inbreeding depression, but that the stress from competition over restricted food among family members (sibling competition and parent–offspring competition over restricted food resources) only had limited effects in the European earwig, *F. auricularia*. In particular, we found that the presence of the mother did not influence the developmental rate of inbred nymphs, whereas it significantly reduced the one of noninbred nymphs. Conversely, brood size did not influence the expression of inbreeding depression in offspring both in terms of developmental or survival rates. These limited effects of inbreeding depression measured in the young offspring contrast to the ones we found when the offspring became adults. In particular, we showed that inbreeding dramatically reduced the reproductive success of male adult offspring (but had only marginal effects on the reproductive success of inbred female adult offspring). Hence, inbreeding depression is sex-specific and more expressed in the late than the early life stages in earwigs.

The benefits associated with maternal presence during family life are commonly thought to be strong enough to buffer against severe forms of inbreeding depression in offspring (e.g. Avilés & Bukowski, 2006; Thünken *et al.*, 2007). Although maternal presence has been shown to provide benefits to earwig offspring reared under natural conditions and under *ad libitum* food in the laboratory (Kölliker, 2007; Kölliker & Vancassel, 2007), maternal presence is also known to reduce offspring developmental and survival rates under limited food condition, possibly due to a mother–offspring competition over the scarce food (Meunier & Kölliker, 2012b). Hence, we hypothesized that under the latter conditions, mother–offspring competition should exacerbate inbreeding depression in earwig offspring. Our results first confirmed that maternal presence reduced developmental and survival rate of outbred nymphs under restricted food conditions and, more surprisingly, revealed that such presence decreased the rate of morphological malformation in (both inbred and outbred) newly produced adults. This finding demonstrates that maternal presence under limited food conditions is also associated with detectable benefits for the offspring. By contrast, we found that the presence or absence of a tending mother did not shape the developmental rate of the inbred nymphs under restricted food conditions. This result is contrary to the prediction that mother–offspring competition exacerbates inbreeding depression, as it suggests that inbreeding may benefit to offspring (in terms of development) by reducing the costs of mother–offspring competition over the scarce, but essential, resources. Further studies exploring the behavioural mechanism mediating this differential effect of maternal presence on inbred versus noninbred offspring, as well as the fitness benefits associated with developmental time in

Table 3 Influence of adult inbreeding status, maternal presence, brood size and total number of adult produced on clutch sex ratio and on the mean weight of males and females. Statistics obtained using GLMMs.

	Clutch sex ratio		Mean weight of males		Mean weight of females	
	LR χ^2_1	*P*-value	LR χ^2_1	*P*-value	LR χ^2_{f1}	*P*-value
Total adult produced	0.63	0.428	1.35	0.245	6.97	**0.008**
Inbreeding status	1.25	0.264	1.89	0.170	1.65	0.199
Maternal presence	0.79	0.376	<0.01	0.925	3.30	0.069
Experimental brood size	2.53	0.112	0.15	0.695	<0.01	0.926
Inbreed. stat.: Maternal presence	1.56	0.212	1.66	0.198	0.85	0.356
Inbreed. stat.: Exp. brood size	0.25	0.621	0.98	0.322	<0.01	0.933

*The significant *P*-value is in bold. LR, likelihood ratio.

Table 4 Sex-specific effect of inbreeding. *P*-values were obtained using randomized GLMs.

	Female offspring *P*-value	Male offspring *P*-value	Interaction *P*-value
First clutch			
Egg number	0.450	0.584	0.785
Hatching success	0.353	**0.007**	0.330
Newly hatched nymphs	0.407	**0.023**	0.460
Mean nymph weight	**0.049**	0.434	0.676
Second clutch			
Egg number	**0.043**	**0.028**	0.459
Hatching success	0.741	0.114	0.521
Newly hatched nymphs	0.215	0.122	0.523
General			
Likelihood 2nd clutch production	0.104	0.222	0.933
Relative investment in 2nd clutch	0.081	0.078	0.622
Total egg number	0.117	0.114	0.566
Total nymph number	0.323	**0.030**	0.453

*Significant *P*-values are in bold.

earwigs will be required. Together with the result that sibling competition over resources did not show stronger effects of inbreeding on nymph development and survival, these findings indicate that family life has comparably weak effects on the expression of inbreeding depression in *F. auricularia* offspring.

The stronger inbreeding depression found in adult compared with young offspring suggest that inbreeding history has purged the most damaging recessive alleles (which typically shape the early life stage) from the studied population (Husband & Schemske, 1996; Glémin, 2003). This inbreeding history is unlikely to result from the laboratory rearing of the field-sampled parents, as these individuals were maintained for only one generation in a very large experimental population (1200 individuals), where they were allowed to freely mate with a large number of unrelated partners. By contrast, some degree of recurrent inbreeding is possible in natural populations of *F. auricularia*, as adults are gregarious and exhibit a relatively low dispersal rate (Moerkens *et al.*, 2010). Further molecular studies (e.g. using microsatellites) exploring the relatedness between group-living adults and the genetic structure of the studied population would help to better understand the evolutionary processes that limited the detrimental effects of inbreeding on nymph survival and development.

Sib mating substantially reduced the reproductive success of earwig male offspring, but had only little effects on the one of female offspring. This male-specific effect of inbreeding depression is in line with recent findings in seed-feeding beetle and fruit flies (Enders & Nunney, 2010; Fox *et al.*, 2011; Okada *et al.*, 2011), but contrasts with results in the flour beetle, *Tribolium castaneum*, where eight generations of inbreeding in the absence of male–male competition did not decrease correlates of male fertility (Michalczyk *et al.*, 2010). Spermatogenesis is commonly known to be particularly vulnerable to inbreeding depression and could thus mediate the male-specific effect of inbreeding observed in *F. auricularia*. One negative effect of inbreeding known to affect spermatogenesis is to decrease the number of sperm in the ejaculates of inbred males (e.g.

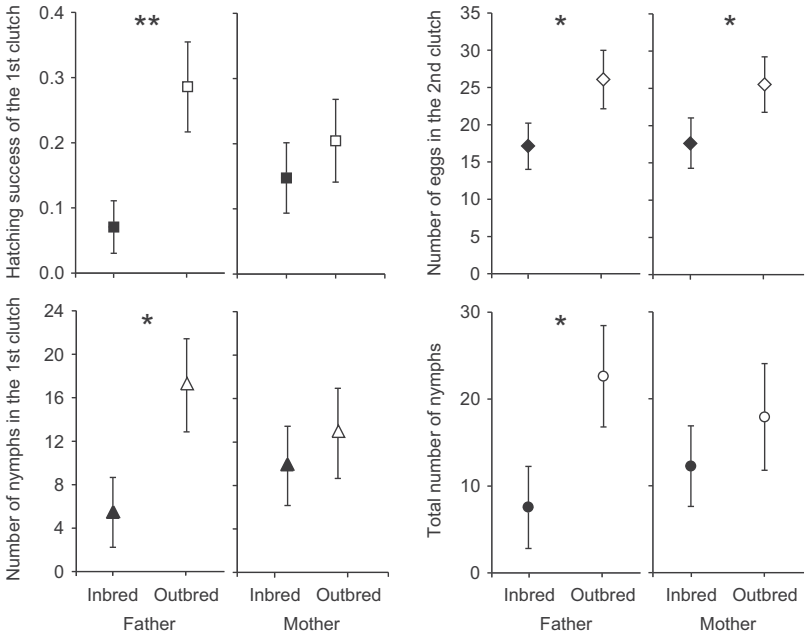

Fig. 3 Sex-specific effects of inbreeding. Adults were originating from sib mating (inbred) or control (outbred) clutches. Values are presented ± SE. $*P < 0.05$, $**P < 0.01$

Zajitschek *et al.*, 2009; Fox *et al.*, 2011) and consequently reduce the number of eggs produced by the mating partners (Fox *et al.*, 2011; Okada *et al.*, 2011). Another potential negative effect is to reduce the quality of sperm in the ejaculates of inbred males (e.g. in terms of viability, abnormal shapes), which hampers embryonic development, lowers the hatching success of the eggs produced by females (e.g. Mehlis *et al.*, 2012), as well as reduces the number of eggs that females are able to produce after having stored the sperm for a relatively long time (due to shorter sperm viability). Although our study did not directly estimate the quantity and quality of sperm produced in earwigs male ejaculates, our findings are in line with lower sperm quality in inbred males. In particular, the hatching success of first clutch eggs sired by inbred males was substantially lower than the one sired by noninbred males. Furthermore, females mated to inbred males produced a smaller number of eggs in their second clutch, which was laid on average 2 months after the production of the first clutch and without remating events in between.

The negative effects of sib mating on the reproductive success of male offspring raise important questions on the influence of inbreeding on the evolution of mating strategies in the European earwig. In particular, the cost of producing inbred adults (sons in particular) could select individuals to actively avoid mating with genetically related partners (sib mating avoidance; e.g. Lihoreau *et al.*, 2008) or females to favour the sperm of unrelated mating partners to fertilize their eggs (e.g. Firman & Simmons, 2008; Welke & Schneider, 2009). Conversely, reduction in the reproductive success of females mating with inbred males could favour mechanisms allowing females to actively choose to mate with noninbred mating partners (e.g. Ilmonen *et al.*, 2009; Okada *et al.*, 2011). To date, the mating strategies of the European earwig are poorly known, beside that males and females have multiple mating partners (Guillet, 2000) and that male mating success is associated with forceps size (Tomkins & Simmons, 1998; Walker & Fell, 2001). Whether inbreeding avoidance, cryptic female choice and/or mate choice against inbred males occur in *F. auricularia* should thus be the aim of future experiments.

Overall, our results reveal that family life has limited effects on inbreeding depression in earwig offspring, a result which is contrary to a common assumption that maternal presence buffers the negative effects of inbreeding on offspring traits (Margulis, 1997, 1998; Avilés & Bukowski, 2006; Thünken *et al.*, 2007). Our study also demonstrates that inbreeding effects measured during the juvenile period underestimates the total fitness costs of sib mating in terms of number of grand-offspring, which emphasizes the key role of long-term studies to better understand the effects of inbreeding on individual fitness (e.g. Cornell & Tregenza, 2007). Finally, our results showed that females exhibit a lower reproductive success when mating with inbred (and unrelated) males than outbred males. To date, few studies disentangled the influence of male and female inbreeding status on the reproductive success of females (Enders & Nunney, 2010; Michalczyk *et al.*, 2010; Fox *et al.*, 2011; Okada *et al.*, 2011; Mattey *et al.*, 2013), possibly because inbred individuals can restore outbred fitness by mating with unrelated partners (and thus regenerating heterozygosis in the offspring; Frankham *et al.*, 2002) (but see Lehmann *et al.*, 2007). The results of the present study in earwigs and the growing number of results in other species (Fox *et al.*, 2011; Okada *et al.*, 2011; Mattey *et al.*, 2013) now emphasizes that mating with inbred individuals (here males) have continued fitness consequences independent from offspring heterozygosity and thus call for more consideration in future theoretical models and empirical studies.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Association between the mean number of adult offspring and the mean weight of female and male offspring.

Table S1 Summary of the sex-specific effects of inbreeding on the reproductive success of offspring.

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When earwig mothers do not care to share: parent-offspring competition and the evolution of family life

Running title: Parent-offspring competition and family life

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SUMMARY

1. Kin competition often reduces – and sometimes entirely negates – the benefits of cooperation among family members. Surprisingly, the impact of kin competition on the fitness effects of family life only received close scrutiny in studies on sibling rivalry, whereas the possiblity of parent-offspring competition has attracted much less attention. As a consequence, it remains unclear whether and how parent-offspring competition could have affected the early evolution of parental care and family life.

2. Here, we examined the occurrence and consequences of parent-offspring competition over food access in the European earwig *Forficula auricularia*, an insect with facultative family life reminiscent of an ancerstral state. Specifically, we (i) raised earwig offspring under food limitation either together with or without their mother, and (ii) tested whether and how the – potentially competitive – weight gains of mothers and offspring during family life affected the offsprings' survival rate and morphology, and the future reproductive investment of their mother.

3. In line with the occurrence of local parent-offspring competition over food access, we showed that high maternal weight gains during family life reduced the survival prospects of maternally tended offspring, while they increased the mothers' investment into the production of a second clutch (but not the body size of the surviving offspring). Conversely, high offspring weight gains generally increased the offsprings' survival, but did so to a larger extent when they were together with their mother. Intriguingly, mothers that had exhibited a low initial weight showed especially high weight gains.

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4. Overall, our results demonstrate that maternal presence under food restriction triggered a local competition between mothers and their offspring. This competition limited offspring survival, but allowed mothers to increase their investment into future reproduction and/or to maintain their current body condition. On a general level, our findings reveal that parent-offspring competition can counteract the benefits of (facultative) parental care, and may thus impede the evolution of family life in resource-poor environments.

Keywords: European earwig; environmental conditions; family life; kin competition; orphaning; parental care; parent-offspring competition; parent-offspring conflict.

INTRODUCTION

The association of offspring with their parents after birth or hatching (defined as family life; Mock & Parker 1997) is a rare, but taxonomically widespread phenomenon (Royle, Smiseth & Kölliker 2012) that can only evolve if the fitness benefits of family interactions outweigh the costs of a prolonged association of the family members (Alonso-Alvarez & Velando 2012). The fitness benefits of family life predominantly derive from the expression of parental care (Costa 2006; Wong, Meunier & Kölliker 2013), which ultimately increases the survival and/or quality of tended juveniles (Klug, Alonso & Bonsall 2012). By contrast, the costs of family life typically result from kin competition among family members for limited resources and reproduction (Krause & Ruxton 2002; West, Pen & Griffin 2002). This competition is known to affect crucial life-history traits such as sex allocation (Frank 1985) and aggressive behavior (West *et al.* 2001), and commonly arises during family life where

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juveniles strive to monopolize limited parental resources (sibling rivalry; Mock & Parker 1997; Roulin & Dreiss 2012). However, kin competition during family life can also arise between offspring and their parents. Such parent-offspring competition can, for instance, promote the dispersal of offspring that reached nutritional independence (Cockburn 1998; Cote, Clobert & Fitze 2007), and therefore hampers the evolution of complex family systems in cooperative breeders (Sorato, Griffith & Russell 2016). Shedding light upon the parties engaged in competition is hence crucial to advance our understanding of the cost-benefit ratio of family life, and more generally its emergence and maintenance in nature (Klug & Bonsall 2010; Klug *et al.* 2012).

The emergence of family life marks the appearance of facultative forms of (post-hatching) parental care (Smiseth, Darwell & Moore 2003; Kölliker 2007). Somewhat surprisingly, the possibility of local parent-offspring competition in species featuring such facultative care has been poorly explored, and its impact on the early evolution of parental care and family life therefore remains unknown. This oversight conceivably results from a historical bias towards studying parental care in altricial – i.e. obligatory-caring – species (Clutton-Brock 1991), in which the scope for parent-offspring competition during early family life is limited by the low foraging capabilities of juveniles. During earlier stages of the evolution of family life (and in contemporary precocial species), however, juveniles are not fully dependent on parental resources (Smiseth *et al.* 2003; Kölliker 2007), and the early onset of offspring foraging might put them into competition with their caring parents early on. In such situations, the costs of parent-offspring competition could diminish the benefits of parental care, and might ultimately render parental presence (Scott & Gladstein 1993; Boncoraglio & Kilner 2012) and family life maladaptive altogether (Meunier & Kölliker 2012).

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The intensity of competition typically depends on the environmentally determined availability of limited resources. Harsh environments could therefore have a profound impact on parent-offspring competition and the evolution of family life (Wilson 1975; Klug *et al.* 2012). The nature of this impact, however, remains controversial. On the one hand, harsh conditions should favor the evolution of family life, because the benefits of parental care are then likely substantial (Wilson 1975; Clutton-Brock 1991). On the other hand, harsh environments are expected to exacerbate the costs of care (such as an increased energy loss; Alonso-Alvarez & Velando 2012), and the limited resource availability may not only favor the deferral of parental investment (Clutton-Brock 1991; Klug & Bonsall 2007), but also increase parent-offspring competition and thus hamper the evolution of family life. In line with this hypothesis, the prolonged presence of fathers has been shown to reduce offspring survival under food limitation in *Nicrophorus vespilloides* Herbst, a burying beetle with biparental care in which both parents feed on the carcass employed for breeding (Scott & Gladstein 1993; Boncoraglio & Kilner 2012). Moreover, food restriction has been shown to offset the benefits of maternal care in the European earwig *Forficula auricularia* L. (Meunier & Kölliker 2012), suggesting that kin competition between offspring and their tending mother might have rendered maternal presence and hence family life detrimental to offspring survival.

In this study, we investigated the occurrence and consequences of mother-offspring competition under harsh conditions (emulated by food restriction) in the European earwig *F. auricularia*. In this precocial insect, mothers provide extensive forms of care to their mobile offspring (called nymphs) for several weeks after hatching (Lamb 1976a). Maternal care includes the protection and grooming of nymphs, as well as their provisioning with food (e.g.

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through regurgitation; Lamb 1976b; Staerkle & Kölliker 2008), and is even expressed by mothers in a bad nutritional condition (Kramer & Meunier 2016). However, post-hatching maternal presence and care are not obligatory for offspring survival (Kölliker 2007; Kölliker & Vancassel 2007), as nymphs can forage independently soon after hatching (relying on the same food resources than their mother; Lamb 1976b) and may even obtain food from their siblings (Falk *et al.* 2014; Kramer, Thesing & Meunier 2015). Consequently, the cost-benefit ratio of maternal presence in *F. auricularia* generally depends on the environmental conditions experienced by mothers and nymphs during family life (Kölliker 2007; Meunier & Kölliker 2012; Thesing *et al.* 2015).

To assess the occurrence of mother-offspring competition, we raised nymphs under food restriction either together with or without their mother, and investigated whether offspring survival was reduced when mothers consumed food and thus restricted offspring feeding (or *vice versa*). We then examined whether the condition of mothers and/or their nymphs at hatching determined the intensity of the putative competition, as well as whether this intensity affected the mothers' future reproduction and the morphology of their (surviving) first-brood offspring. If mother-offspring competition occurred, we would expect that maternal weight gains during family life negatively affect offspring survival under maternal presence (but not absence). Moreover, we predicted that mothers exhibiting a low initial weight would compete more intensely – and thus curtail their offspring's survival more extensively - than mothers exhibiting a high initial weight. Similarly, we expected that groups of nymphs with low initial weight would compete more intensely with their mother to ensure their own survival. Finally, we predicted that intense competition might benefit

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mothers and the surviving offspring, for instance by enabling them to increase their investment into future reproduction and their final body size, respectively.

MATERIALS AND METHODS

Laboratory rearing and experimental design

We investigated the occurrence and potential repercussions of mother-offspring competition under harsh conditions in 128 female earwigs and their first brood. The females had been collected in July and August 2013 in Mainz, Germany and were reared under standard laboratory conditions (adapted from Meunier *et al.* 2012; see Appendix S1 in Supporting Information) until they produced their first clutch. On the first day after egg hatching, we haphazardly distributed a random subset of 32 nymphs (original brood size: 49.6 ± 1.1 nymphs; mean ± se) among two equally sized groups. We manipulated the potential for mother-offspring competition over food access by raising one of these groups together with their mother (maternal presence-, or *MP-group*) and the other group without their mother (maternal absence-, or *MA-group*). After 16 days, mothers were removed from the MP-groups to mimic natural family disruption (Meunier *et al.* 2012) and to determine their investment into the production of a second (and final) clutch. In parallel, the MP- and MA-nymphs were maintained in their groups, and their survival and morphology were measured upon adult emergence. Note that we excluded 16 of the 128 initially employed families from our analyses because the mother died during family life.

Harsh environmental conditions were emulated according to an established protocol (Meunier & Kölliker 2012) by providing MP- and MA-groups with a restricted amount of an artificial diet (composition detailed in Kramer *et al.* 2015) every six days for the duration of

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three days, respectively. This six-day cycle was initiated on the first day after hatching and repeated until the juveniles reached adulthood. The amount of food the groups received at a time was increased stepwise from 60 mg (until the end of family life) to 120 mg (until day 31) and finally to 240 mg (until adulthood; cf. Meunier & Kölliker 2012). This feeding regime successfully established scope for mother-offspring competition, as the food provided during family life was more often fully consumed before removal in MP- than in MA-groups (in 81 vs. 25 % of cases; paired Wilcoxon signed rank test: $V = 4656$, $P > 0.0001$). We also manipulated the conditions experienced by mothers after family life to investigate the effect of food availability during that period on their investment into a second clutch. To this end, isolated mothers either received 60 mg of food in a continuation of the above detailed six-day cycle (low food-treatment; n = 54 mothers), or an *ad libitum* amount that was renewed twice per week (high food-treatment; n = 58 mothers). Isolated mothers and all groups of young nymphs were kept in medium Petri dishes (Ø = 9 cm) throughout the experiment. On day 31, groups of older nymphs were transferred into large Petri dishes (Ø = 14 cm). All Petri dishes contained humid sand as substrate and a plastic tube as shelter.

Measurements

We determined the occurrence and intensity of mother-offspring competition by testing for the occurrence and strength of a negative association between maternal food consumption and offspring fitness. To this end, we measured the relative weight gains of mothers and nymphs during family life (as a proxy of food intake), and gathered the survival rate of nymphs at the end of family life and upon adult emergence in all remaining 112 families (see above). The weights of mothers were determined by weighing each mother on the first day

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after egg hatching and at the end of family life. Similarly, the average weights of nymphs were determined by weighing all nymphs of a family on the first day after hatching, as well as all surviving nymphs of a group at the end of family life, and then dividing these weights by the corresponding number of weighed nymphs. The *relative* weight changes were calculated by subtracting the weight at the beginning of family life from the corresponding weight at its end, and then dividing this difference by the initial weight. All mothers and nymphs were weighed to the nearest 0.01 mg using a microscale (MYA5, PESCALE, Bisingen, Germany). Offspring survival rates at the end of family life were determined by counting the nymphs that survived until day 16, and then dividing this number by the number of nymphs initially distributed to that group. Likewise, the survival rates upon adult emergence were determined by counting the nymphs that survived until adulthood, and then dividing this number by the number of nymphs alive at the end of family life. Note that two nymphs per group were removed six days after hatching to conduct an independent experiment (data not shown). These nymphs were not considered in the calculation of survival rates.

We also determined the consequences of the suspected mother-offspring competition for the future (and final) reproductive effort of mothers, as well as for the morphology of the surviving, adult offspring. To this end, we checked all 112 isolated mothers daily for oviposition over a period of 100 days, and assessed (1) the occurrence of egg deposition and - where applicable - (2) the length of the inter-clutch interval and (3) clutch size (the number of eggs produced within three days after the onset of egg-deposition; Meunier & Kölliker 2013). The inter-clutch interval was defined as the number of days between isolation from the first brood and the deposition of the second clutch (Kölliker 2007). The morphology of adult offspring was assessed by measuring two fitness-relevant

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morphological traits – eye distance (a proxy of body size) and forceps length (Radesäter & Halldórsdóttir 1993) – in the first male and female adult that emerged in each group. Overall, we measured 192 males and 198 females [at least one male emerged in 92 (100), and at least one female in 94 (104) of the 112 MP- (MA-) groups]. All morphological measurements were taken under CO_2-anesthetization to the nearest 0.001 cm using a camera coupled to a stereo microscope (DFC425, Leica Microsystems Ltd, Heerbrugg, Switzerland) and operated with the software *Leica Application Suite 4.5.0.*

Statistical analyses

Establishing the occurrence of mother-offspring competition over food access requires to demonstrate that offspring fitness under maternal presence is reduced (and maternal fitness increased) when mothers restrict offspring feeding through their own food consumption (or vice versa). Accordingly, we predicted that high maternal weight gains would (1) only impair the survival and/or morphology of offspring raised under maternal presence, and (2) increase maternal investment into the production of a second clutch. To verify our first prediction, we used a generalized linear mixed model (GLMM) with binomial error distribution to test whether offspring survival rate (entered as odds ratio) was affected by offspring and maternal weight gains, maternal presence (MP or MA), and the observation period (from the beginning until the end of family life or from the end of family life until adult emergence). An observation-level variable and Family-ID were entered into the model as random effects to account for overdispersion (Harrison 2015) and the common origin of nymphs in the MP- and MA-groups, respectively. Because a four-way-interaction between all explanatory variables shaped offspring survival (Table S1; Wald χ_1^2 = 7.80, P = 0.0052), we

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split the data set by the observation period and fitted two GLMMs with the same set of variables (except the observation period) within each subset. Similarly, we fitted two separate linear mixed models (LMMs) using Family-ID as a random effect to test whether either the eye distance or the forceps length (corrected for eye distance; details in Appendix S2) of adult offspring was affected by maternal and offspring weight gains, maternal presence, and adult sex.

To examine our second prediction, we tested in two generalized linear models (GLMs) fitted with a binomial error distribution (corrected for overdispersion) and one linear model (LM) whether the weight gains of mothers and/or offspring affected the mothers' future reproduction. Each of these models included maternal and offspring weight gains, as well as the availability of food after family life (HF or LF) as explanatory variables. The GLMs were fitted using either the occurrence of 2nd clutch production or the relative investment of mothers into the production of their 2nd clutch (entered as odds ratio of 2nd to 1st clutch eggs) as a response variable. Conversely, the LM was fitted using the length of the inter-clutch interval as response variable. Note that this LM and the GLM analyzing the relative investment of mothers into their 2nd clutch were fitted on the subset of those mothers that eventually produced a second clutch. In the final step of our analysis, we investigated the determinants of maternal and offspring weight gains. To this end, we fitted one LM and one LMM that each used the weight of the mother and of the nymphs at hatching (corrected for the initial weight of the mother; details in Appendix S3) as explanatory variables. The LM and LMM were fitted using maternal weight gain and offspring weight gain as response variable, respectively. Because this latter model was fitted on both MP- *and* MA-groups, we included Family-ID as a random effect.

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All statistical analyses were performed with the statistics software R version 3.0.3 (http://www.r-project.org/). Mixed model analyses were implemented using the packages *lme4, car*, and *lmerTest*. Note that we scaled the 'relative weight gain of mothers' and the 'average relative weight gain of the offspring' to unit variance to avoid model bias due to collinearity between these variables (variance inflation factor < 4.0 after scaling in all models). All statistical models initially included all possible interactions between the tested variables and were then simplified via the stepwise deletion of non-significant interactions (all $p < 0.05$). Interactions between continuous variables were plotted using the package 'effects' to display the predicted relationship between the response variable and one explanatory variable for different values of the interacting variable(s) (details in Fox 2003).

RESULTS

Two independent interactions between maternal presence and, respectively, the relative weight gains of mothers and the relative weight gains of the offspring determined offspring survival until adulthood (Table 1a). In line with the occurrence of mother-offspring competition, the first interaction revealed that high maternal weight gains reduced the long-term survival of offspring when the mother was present (Fig.1a; estimate ± se = -0.175 ± 0.070, z = -2.518, P = 0.0118), but not when she was absent during family life (Fig.1a; estimate ± se = 0.047 ± 0.067, z = 0.698, P = 0.4854). Conversely, the second interaction showed that the offsprings' long-term survival increased more steeply with their weight gains under maternal presence (Fig.1b; maternal presence: estimate ± se = 0.359 ± 0.072, z = 4.983, P < 0.0001; maternal absence : estimate ± se = 0.161 ± 0.067, z = 2.396, P = 0.0166).

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Offspring survival until the end of family life was shaped by a triple-interaction between maternal presence and the weight gains of mothers and their offspring (Table 1b). In particular, high maternal weight gains reduced the positive impact of high offspring weight gains on offspring survival under maternal presence (Fig.2; maternal weight gain: χ_1^2 = 1.083, P = 0.2981; offspring weight gain: χ_1^2 = 0.083, P = 0.7736; interaction: χ_1^2 = 8.278, P = 0.0040). By contrast, offspring survival during family life was independent of both maternal and offspring weight gains under maternal absence (interaction: χ_1^2 = 2.588, P = 0.1077; maternal weight gain: χ_1^2 = 0.276, P = 0.5995; offspring weight gain: χ_1^2 = 0.592, P = 0.4416).

The weight gains of mothers and their offspring during family life overall decreased with their own initial weight (weight gain of mother : Fig.3a, estimate ± se = -7.202 ± 1.081, t = -6.663, P < 0.0001; weight gain of offspring : Fig.3b, estimate ± se = -0.091 ± 0.022, $t_{112.00}$ = -4.164, P < 0.0001), but were independent of each other's initial weight (weight gain of mother : estimate ± se: -62.058 ± 48.345, t = -1.284, P = 0.2020; weight gain of offspring : estimate ± se: 1.685 ± 3.157, $t_{112.00}$ = 0.534, P = 0.5940), respectively. Notably, offspring collectively gained less weight under maternal presence (mean ± se: 0.86 ± 0.03) than under maternal absence (0.99 ± 0.03; χ_1^2 = 23.478, P < 0.0001).

Overall, 61 of the 112 mothers (54.5 %) produced a second clutch. Both the likelihood of 2nd clutch production (Table 2a) and the length of the inter-clutch interval (Table 2b) were shaped by an interaction between maternal and offspring weight gains during 1st brood family life. Intriguingly, these interactions indicated that high maternal weight gains increased the likelihood of 2nd clutch production (Fig.4a) and decreased the length of the inter-clutch interval (Fig.4b) only if the 1st brood offspring had featured high weight gains. By contrast, high maternal weight gains actually reduced the likelihood of 2nd clutch production

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(Figure 4a), and increased the inter-clutch interval (Fig.4b), if they occurred in combination with low offspring weight gains. Note that the interactive effect of maternal and offspring weight gains on the length of the inter-clutch interval was contingent upon the mothers' access to food after family life, as the interaction only emerged when food was restricted (interaction: F_1 = 6.03, P = 0.0234; maternal weight gain: F_1 = 0.11, P = 0.7458; offspring weight gain: F_1 = 0.01, P = 0.9364), but not when it was provided *ad libitum* (interaction: F_1 = 2.68, P = 0.1112; maternal weight gain: F_1 = 0.18, P = 0.6742; offspring weight gain: F_1 = 0.12, P = 0.7325). Finally, the investment into 2nd clutch production increased with maternal weight gains during 1st brood family life (Table 2c; estimate ± se: 0.228 ± 0.098, t = 2.322, P = 0.0238), and was overall higher when mothers had received food *ad libitum* after family life (Table 2c). By contrast, the weight gains of 1st brood offspring did not affect the relative investment of mothers into their 2nd clutch (Table 2c; estimate ± se: -0.150 ± 0.104, t = -1.453, P = 0.1516).

Maternal presence affected the eye distance, but not the corrected forceps length, of those offspring that survived until adulthood. In particular, the eye distance of adult offspring was overall smaller when they grew up together with (mean ± SE = 1.340 ± 0.005 mm) rather than without their mother (1.354 ± 0.005 mm; Table 3a), and overall larger in females (1.370 ± 0.005 mm) than in males (1.324 ± 0.004 mm; Table 3a). Moreover, eye distance decreased with increasing maternal weight gains irrespective of maternal presence (Table 3a, estimate ± se: -0.011 ± 0.004, $t_{102.20}$ = -3.137, P = 0.0022), but was independent of offspring weight gains (Table 3a). By contrast, the corrected forceps length was overall larger in males (0.240 ± 0.017 mm) than in females (-0.233 ± 0.012 mm), but independent of

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maternal presence and the weight gains of the offspring and their mother during family life (Table 3b).

DISCUSSION

Kin competition often reduces and sometimes even entirely negates the benefits of cooperation among relatives (West *et al.* 2002). Surprisingly, however, the role of kin competition between parents and their offspring in the early evolution of parental care has been largely overlooked. Here, we demonstrated that maternal presence under food restriction triggered a local mother-offspring competition in the European earwig *F. auricularia*. This competition manifested itself as a negative effect of high maternal weight gains on the survival of maternally tended (but not maternally deprived) offspring, and positively affected the future reproductive investment of tending mothers. Our results also reveal that the extent of maternal weight gains – and thus the intensity of mother-offspring competition – was highest when mothers had featured a low weight at egg hatching. Finally, we found that maternal presence curtailed the adult body size of those offspring that overcame the lethal consequences of the competition with their mother.

We showed that high maternal weight gains during family life reduced offspring survival until adulthood. Notably, this negative effect (1) was only present in offspring that grew up *with* their mother, (2) arose *after* the end of (i.e. was not detectable during) family life, and (3) was paralleled by a generally lower weight gain of maternally-tended (as compared to maternally-deprived) offspring during family life. Together, these findings demonstrate that mothers competed with their offspring by consuming portions of the limited amount of food available, and thereby indirectly limited the long-term survival

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prospects of their progeny, e.g. by increasing the likelihood of offspring starvation or by triggering high levels of siblicide (Dobler & Kölliker 2010). These findings are unlikely to result from the prevention of offspring dispersal in our experimental setup, since nymph dispersal in *F. auricularia* is not accelerated by food limitation (Wong & Kölliker 2012). Hence, our findings emphasize that local competition between parents and their offspring might counteract the benefits of facultative care (Scott & Gladstein 1993; Ward, Cotter & Kilner 2009; Boncoraglio & Kilner 2012) and thus impede the transition to – and the maintenance of – social life in resource-poor environments.

Interestingly, maternal and offspring weight gains interacted in determining the survival of maternally-tended offspring *during* family life: the association between offspring weight gains and survival rates shifted from positive to negative when maternal weight gains were low and high, respectively. This suggest that high offspring weight gains are beneficial when mother-offspring competition is moderate (i.e. when maternal weight gains are low), but costly when it is intense (i.e. when maternal weight gains are high). The first pattern would then indicate that the offsprings' survival mostly relies on their own quality (as reflected by their weight gains) when their mother does not monopolize the food resources. Conversely, the second pattern would indicate that high levels of mother-offspring competition result in a lower amount of left-over food per offspring, which could in turn trigger higher levels of sibling rivalry and siblicide (Dobler & Kölliker 2010; Gardner & Smiseth 2011; Wong, Lucas & Kölliker 2014). Alternatively, this second pattern could reflect filial cannibalism, for instance aimed at increasing the survival prospects of the remaining juveniles (Forbes & Mock 1998; Klug & Bonsall 2007). In both cases, the resulting lower overall offspring survival would increase the *per-capita* amount of food available to – and

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thus the maximum weight gain achievable by – the remaining (high-quality) juveniles and their mother. The exact mechanism notwithstanding, our results show that maternal presence under food limitation can reduce offspring fitness even in the short run (see also Thesing *et al.* 2015).

We showed that the initial weight of the mothers, but not the initial weight of their offspring, affected the intensity of mother-offspring competition. Mothers with a low weight at egg hatching gained more weight at the expense of their offspring – and thus reduced their offspring's survival more substantially – than initially heavier mothers. Importantly, maternal weight gains did not differ between clutches of particularly heavy and light nymphs, suggesting that mothers did not adjust the extent of competition in relation to the perceived reproductive value of their offspring (Kilner & Hinde 2012). Together with the observation that offspring overall gained less weight under maternal presence, these findings indicate that the extent of mother-offspring competition is subject to maternal control (Smiseth, Wright & Kölliker 2008; Hinde, Johnstone & Kilner 2010). Note that this holds true although initially heavy nymphs showed lower relative weight gains than initially light nymphs. This is because the above relationship arose independent of maternal presence, indicating that it reflected intrinsic factors such as the genetic quality of family members (Wilson & Nussey 2010) rather than the extrinsic influence of competition. Overall, our findings thus suggest that the (genetic) quality and/or the condition of mothers (see also Koch & Meunier 2014) determine how mothers react to food limitation (e.g. by affecting their responsiveness to offspring solicitation behaviors; Grodzinski & Johnstone 2012) and thus whether they compete with their offspring. Here, mothers of low quality or bad condition might have favored somatic maintenance over self-restraint (McNamara &

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Houston 1996; Alonso-Alvarez & Velando 2012), either to safeguard their ability to perform crucial parenting behaviors such as predator defense (Bateson 1994), or in an attempt to shift their investment from current to future offspring (Thorogood, Ewen & Kilner 2011; Kramer & Meunier 2016).

In line with the hypothesis of a condition-dependent shift of maternal investment, we found that high maternal weight gains – and thus intense mother-offspring competition – increased the relative investment into 2nd clutch production among mothers that produced another clutch. Conversely, high maternal weight gains promoted or inhibited the likelihood of 2nd clutch production depending on whether these gains had occurred in concert with high or low offspring weight gains during 1st brood family life, respectively. Finally, we found that the same pattern shaped the length of the inter-clutch interval, but only among mothers that had received a restricted (rather than an *ad libitum*) amount of food after the isolation from their 1st brood offspring. Overall, these findings illustrate that mother-offspring competition might not always trigger a shift in maternal investment from current to future reproduction (Klug *et al.* 2012). This supports the conjecture of an additional motivation for this competition (e.g. the maintenance of maternal condition to perform crucial parenting behaviors; Bateson 1994), which in turn highlights that parent-offspring competition should not uncritically be taken as evidence for parent-offspring conflict (*sensu* Trivers 1974). In particular, some mothers might have heavily invested into their current (low-quality) offspring, thus giving rise to the negative effect of high maternal weight gains on the likelihood of 2nd clutch production in broods featuring low offspring weight gains. This suggests that the scope for conflict might be limited if mothers re-invest competitively

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acquired resources into their current brood and thereby offset the costs of competition for 1st brood juveniles.

In contrast to its influence on offspring survival and maternal investment into future reproduction, mother-offspring competition did not affect the size and forceps length of offspring that survived until adulthood. This result suggests that this competition likely does not reflect an *indirect* mechanism of adaptive brood reduction by mothers. Specifically, mother-offspring competition does not seem to have handicapped low-quality offspring to an extent that favored the survival and development of higher-quality offspring, as the average size of maternally-tended offspring should have been larger than that of non-tended juveniles in this scenario (Simmons 1988; Mock & Parker 1997). Nevertheless, our results showed that adults were overall smaller when they had been raised with their mother, a negative effect of maternal presence that has already been found under favorable conditions (featuring unrestricted food access) and that might result from maternal behaviors that are maladaptive under laboratory conditions (such as the burying of food, presumably to prevent microbial growth; see Thesing *et al.* 2015). Our results also revealed that males were overall smaller but had longer forceps than females, confirming the sexual dimorphism of these morphological traits (Radesäter & Halldórsdóttir 1993; Thesing *et al.* 2015). Finally, we showed that body size generally decreased with increasing weight gains of mothers during 1st brood family life. This effect arose independent of maternal presence, indicating that it likely reflected the overall lower initial weight (and thus size) of offspring produced by those mothers that gained a lot of weight during family life rather than the extrinsic influence of competition.

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In conclusion, we demonstrated that local competition between parents and their offspring can drastically reduce offspring fitness in species with facultative family life. Parent-offspring competition might thus not only diminish the benefits of a prolonged association of parents with their offspring (Scott & Gladstein 1993), but even impede the evolution of family life altogether. This finding illustrates that parental presence can be associated with costs for the tended offspring (Scott & Gladstein 1993; Meunier & Kölliker 2012; Thesing *et al.* 2015) that are usually masked by the benefits of parenting behaviors in the wild (Costa 2006; Wong *et al.* 2013), but that emerge whenever the (laboratory) conditions prevent these benefits from taking effect. While parents might be able to reduce the costs of local competition with their offspring under natural conditions, the behavioral changes necessary to do so are likely themselves costly. For example, parents might increase their foraging range (West *et al.* 2002), but will then likely suffer from a higher predation risk (Alonso-Alvarez & Velando 2012). Our results thus generally stress the crucial role of environmental factors such as resource availability and predation pressure in the early evolution of social life. Importantly, our findings also provide a diachronic perspective on social evolution: they suggest that competition between parents and their offspring should decline with an increasing reliance of offspring on parentally provided resources, a process that in turn is known to increase the scope for competition *among* offspring (Gardner & Smiseth 2011). The transition from facultative to obligatory forms of family life might hence be accompanied by a shift in the type of competition that most profoundly affects family interactions.

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AUTHORS' CONTRIBUTION STATEMENT. JK and JM conceived the ideas and designed the experiment; JK, MK, JMCD, CS, AYD, TC, and PK collected the data; JK analyzed the data; JK led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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DATA ACCESSIBILITY. Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.651ss (Kramer *et al.* 2017).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1. Rearing protocol

Table S1. Results of the full model analyzing offspring survival

Appendix S2. Correction of forceps length

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Appendix S3. Correction of offspring weight at hatching

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Table 1 | Offspring survival. Influence of maternal presence, and the relative weight gain of mothers and their offspring on the offsprings' survival (a) upon adult emergence and (b) at the end of family life. Significant P-values are given in bold print ([§] values as indicated before removal of the interaction from the model).

	offspring survival			
	(a) upon adult emergence		(b) at the end of family life	
	Wald χ_1^2	P	Wald χ_1^2	P
maternal presence (MP)	51.92	**< 0.0001**	4.67	**0.0306**
offspring weight gain (OWG)	23.61	**< 0.0001**	1.49	0.2228
maternal weight gain (MWG)	1.21	0.2711	< 0.01	0.9735
MP : MWG	4.56	**0.0327**	0.14	0.7085
MP : OWG	6.84	**0.0089**	0.92	0.3383
MWG : OWG	1.56[§]	0.2114[§]	0.02	0.9029
MP: MWG : OWG	2.35[§]	0.1255[§]	7.00	**0.0082**

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Table 2 | Maternal investment into 2nd clutch production. Influence of maternal weight gains, offspring weight gains, and the food availability after family life (high or low food) on (a) the likelihood of 2nd clutch production, (b) the length of the inter-clutch interval, and (c) the relative investment into 2nd clutch production. Significant P-values are given in bold print (§ values as indicated before removal of the interaction from the model).

	(a) likelihood of production		(b) inter-clutch interval		(c) relative investment	
	LR χ_1^2	P	F_1	P	LR χ_1^2	P
maternal weight gain (MWG)	0.01	0.9177	0.28	0.5962	5.39	**0.0203**
offspring weight gain (OWG)	2.03	0.1546	0.05	0.8285	2.18	0.1399
food availability (FA)	3.70	0.0544	4.49	**0.0388**	4.10	**0.0429**
MWG : OWG	10.19	**0.0014**	0.13	0.7172	0.80§	0.3712§
MWG : FA	2.77§	0.0961§	0.02	0.8967	0.03§	0.8607§
OWG : FA	1.11§	0.2921§	0.12	0.7302	1.00§	0.3185§
MWG : OWG : FA	0.48§	0.4904§	7.32	**0.0092**	0.02§	0.8950§
type of model | sample size	GLM | 112		LM | 61		GLM | 61	

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Table 3 | Morphology of the surviving adult offspring. Influence of maternal presence, offspring sex, and the weight gains of nymphs and their mother during family life on (a) the eye distance and (b) the (corrected) forceps length of the surviving adult offspring. Significant P-values are given in bold print ([§] values as indicated before removal of the interaction from the model).

	(a) eye distance		(b) forceps length	
	Wald χ_1^2	P	Wald χ_1^2	P
maternal presence	6.16	**0.0131**	0.69	0.4075
sex	59.51	**< 0.0001**	659.51	**< 0.0001**
maternal weight gain	9.84	**0.0017**	0.69	0.4075
offspring weight gain	0.37	0.5423	1.97	0.1608

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Figure 1 | Offspring survival until adulthood. Influence of (a) the relative weight gain of mothers and (b) the relative weight gain of their offspring on offspring survival until adulthood under maternal presence (filled squares) and absence (empty squares).

Figure 2 | Interactive effect of maternal and offspring weight gains on the survival of maternally-tended offspring during family life. To illustrate the interaction, regression lines are given for an average value of maternal weight gains (median = 0.13; dashed line), as well as for a comparatively low (1st quantile = 0.07; solid line) and high (3rd quantile = 0.20; dotted line) weight gains, respectively.

Figure 3 | Weight gains and initial weight. Influence of (a) the weight of mothers and (b) the weight of nymphs at egg hatching (corrected for the weight of their mother; see above) on their respective weight gains during family life.

Figure 4 | Interactive effects of maternal and offspring weight gains on 2nd clutch traits. Depicted are the interactive effects of maternal and offspring weight gains on (a) the likelihood of 2nd clutch production among all mothers, as well as on (b) the length of the inter-clutch interval among those mothers with restricted food access after family life. To illustrate the interactions, regression lines are given for an average value of offspring weight gains (median = 0.82; dashed line), as well as for a low (1st quantile = 0.64; solid line) and a high value (3rd quantile = 1.03; dotted line), respectively. Note that the axis depicting the inter-clutch interval is reversed as shorter intervals likely reflect higher maternal fitness.

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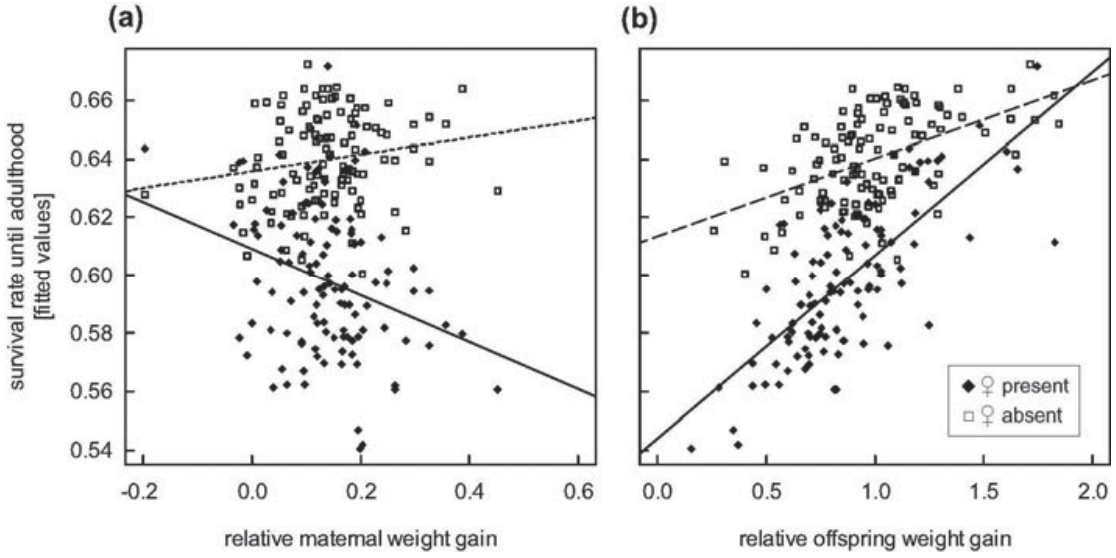

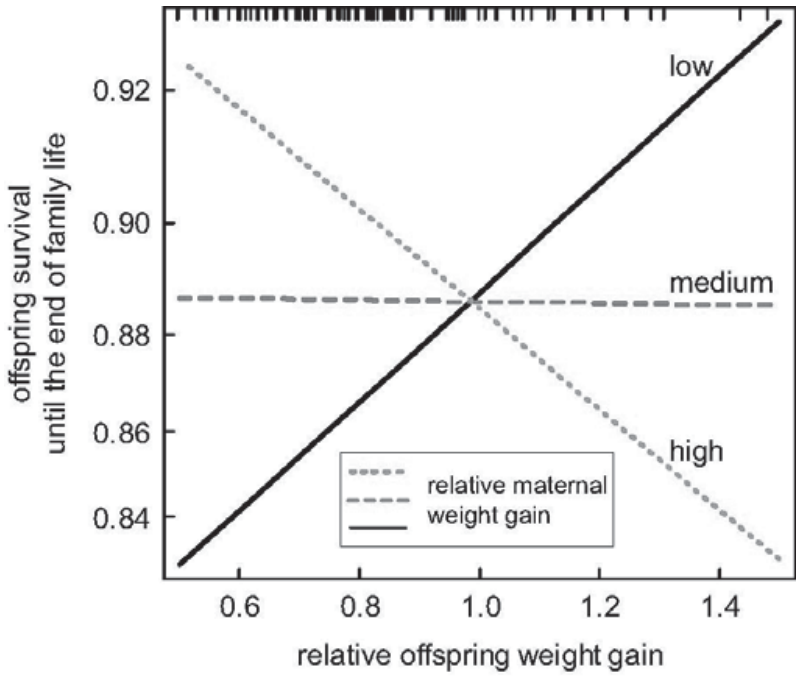

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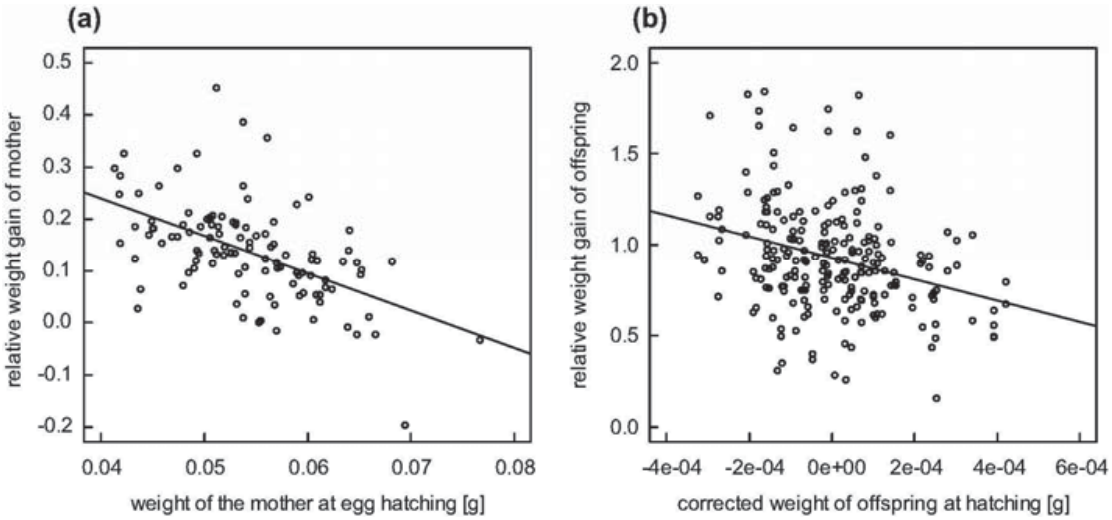

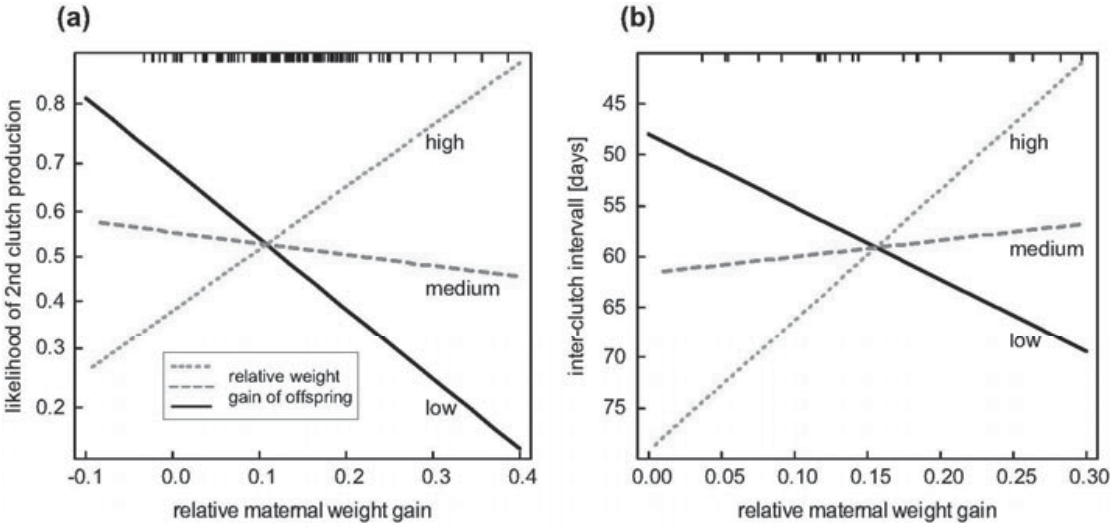

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Sibling Cooperation in Earwig Families Provides Insights into the Early Evolution of Social Life

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abstract: The evolutionary transition from solitary to social life is driven by direct and indirect fitness benefits of social interactions. Understanding the conditions promoting the early evolution of social life therefore requires identification of these benefits in nonderived social systems, such as animal families where offspring are mobile and able to disperse and will survive independently. Family life is well known to provide benefits to offspring through parental care, but research on sibling interactions generally focused on fitness costs to offspring due to competitive behaviors. Here we show experimentally that sibling interactions also reflect cooperative behaviors in the form of food sharing in nonderived families of the European earwig, *Forficula auricularia*. Food ingested by individual offspring was transferred to their siblings through mouth-to-anus contacts and active allo-coprophagy. These transfers occurred in both the presence and the absence of the tending mothers, even though the direct contact with the mothers limited sibling food sharing. Neither food deprivation or relatedness influenced the total amount of transferred food, but relatedness affected frass release and the behavioral mechanisms mediating food sharing. Related offspring obtained food predominately through allo-coprophagy, whereas unrelated offspring obtained food through mouth-to-anus contacts. Overall, this study emphasizes that sibling cooperation may be a key process promoting the early evolution of social life.

Keywords: family life, sibling rivalry, precocial species, food sharing, *Forficula auricularia*.

Introduction

The diversity of social life is characterized by different degrees of complexity ranging from simple gregariousness, family group with parental care to complex societies with reproductive division of labor (Wilson 1971, 1975; Krause and Ruxton 2002; Costa 2006*a*). The ecological success of group living species is commonly attributed to the benefits of social interactions in terms of direct or indirect fitness, such as higher brood survival, more efficient food acquisition, and/or antipredator defenses (Wilson 1975; Krause and Ruxton 2002; Gross 2005; Royle et al. 2012; Smiseth et al. 2012). However, group living can also be associated with major fitness costs, for example, due to enhanced risks of pathogen transmission among group members (Schmid-Hempel 1998) or social conflicts over resources and reproduction (Mock and Parker 1997; Krause and Ruxton 2002; Ratnieks et al. 2006; East and Hofer 2010; Meunier et al. 2010). Hence, social life can only evolve if the associated benefits outweigh the costs (Bourke 2011; Alonso-Alvarez and Velando 2012). Investigating the source of these benefits in derived social systems displaying obligate and/or permanent forms of group living can be misleading if we want to infer the conditions under which group formation and social life emerged and originally evolved (e.g., Smiseth et al. 2003). Instead, gaining a better understanding of the early conditions promoting social living from a solitary state requires identifying the nature of cooperative behaviors expressed in species exhibiting facultative and/or temporary forms of social life.

In obligate and permanent colonies of eusocial insects (e.g., bees, wasps, ants, and termites), cooperative behaviors among offspring are key social processes, during which adult offspring (i.e., workers) are required to actively and frequently share food with their siblings (i.e., other adult workers and larvae; Wilson 1971). By contrast, in the case of simpler and less derived forms of social life, notably in species with parental care (e.g., Trumbo 2012; Wong et al. 2013), sibling interactions are typically characterized by antagonistic behaviors such as competitive begging and siblicide (reviewed in Mock and Parker 1997; Roulin and Dreiss 2012). Whereas this evidence suggests that sibling cooperation in the form of food sharing is a derived trait of more permanent and complex social systems, a few studies in species with parental care showed that siblings competing for parental resources may also express cooperative behaviors under certain conditions (Forbes 2007). For instance, in the barn owls *Tyto alba*, chicks may actively

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share parentally provided prey items with their siblings (Roulin et al. 2012), as well as vocally negotiate with nonstarved siblings about these limited prey items rather than fight over them (Roulin et al. 2000; Roulin 2002; Dreiss et al. 2010, 2013; see also the spotless starling *Sturnus unicolor*; Bulmer et al. 2007). Negotiation is considered as cooperative because it prevents starved offspring from suffering from the costs of intense physical fights while providing indirect fitness benefits to the related cooperative offspring (Johnstone and Roulin 2003). A different form of cooperation occurs when siblings coordinate their begging behavior when parents return from foraging trips, as reported in the black-headed gull *Larus ridibundus* (Mathevon and Charrier 2004) and in the banded mongoose *Mungos mungo* (Bell 2007). This cooperative interaction provides benefits to all contemporary siblings by more effectively increasing parental feeding rate, while limiting each offspring's personal investment in costly begging (Johnstone 2004). Finally, dominant siblings may avoid physical competition with subordinates, as found in the blue-footed booby *Sula nebouxii*, despite their capacity for siblicide, a cooperative restraint possibly driven by kin selection (Anderson and Ricklefs 1995).

The above examples for sibling cooperation are mostly from studies of altricial bird and mammalian species in which the mobility of offspring is limited during the time when parents provide care (i.e., offspring are usually spatially confined to a nest) and their survival mainly relies on the resources provided by the tending parents. While these studies show that various forms of sibling cooperation occur under obligate parental care, they are of limited relevance to understand the role of sibling cooperation in the original evolution of parental care and social life. In particular, it is unknown whether forms of sibling cooperation occur in species where offspring have the capabilities to both actively disperse from their family group and survive in absence of tending parents, the scenario that likely prevailed in the early evolution of social life. Interestingly, early dispersal and independent survival can be found in offspring from precocial species where care is facultative, which are common across taxa (e.g., Smiseth et al. 2003; Rehling and Trillmich 2007; Dial and Carrier 2012) and thus provide ideal biological models to better understand the evolutionary mechanisms that favored the emergence and persistence of parental care and social life.

This study tested the occurrence of sibling cooperation in form of food sharing in families of the European earwig, *Forficula auricularia*. In this insect species, mothers tend their clutch of mobile offspring (called nymphs) for several weeks after the eggs have hatched (Costa 2006*b*). During this period, mothers frequently interact with their nymphs, protect them against predators, and provide food through trophallaxis (Staerkle and Kölliker 2008; Meunier and Kölliker 2012*a*, 2012*b*, 2013). Although tending mothers are known to enhance nymph survival under natural and laboratory conditions (Kölliker 2007; Kölliker and Vancassel 2007), maternal attendance is not required to ensure such survival (Lamb 1976; Kölliker 2007) and may even come at costs to nymph survival when food is limited (Meunier and Kölliker 2012*b*). Nevertheless, earwig nymphs have been shown to actively aggregate with their mothers under both good and harsh conditions (Wong and Kölliker 2012). Besides mother-offspring contacts, there are frequent and tight interactions among nymphs that are known to be partly competitive. For instance, both large clutches and limited food availability have been shown to reduce nymph survival (Kölliker 2007; Meunier and Kölliker 2012*a*), and siblicide and cannibalism are relatively frequent during family life (Dobler and Kölliker 2010). Alternatively, recent observations suggest that nymph-nymph interactions may also take the forms of allo-grooming as well as mouth-to-mouth and mouth-to-anus contacts (J. Meunier, personal observation), which possibly reflect cooperative behaviors.

We conducted a series of three laboratory experiments to address the following four questions. First, do nymphs exchange food during family life, and what is the effect of maternal presence on this exchange? Second, can opportunistic allo-coprophagy (i.e., feeding on frass released by conspecifics) at least partly mediate food transfer among nymphs? Third, is food transfer between siblings an active or a passive process? To this end, we defined stomodeal trophallaxis (mouth-to-mouth feeding), proctodeal trophallaxis (anus-to-mouth feeding) and/or active allo-coprophagy (i.e., resulting from the socially induced release of frass) as active processes, whereas opportunistic allo-coprophagy was defined as passive process. Finally, what are the influences of nymph-nymph relatedness and nymph condition (i.e., food deprivation) on the level of food transfer? These two factors were chosen because relatedness is considered a key parameter in the evolution of cooperation and social systems (Hamilton 1964; West et al. 2002), relatedness between earwig nymphs naturally varies within clutches due to the promiscuous mating system of adults and the phenomenon of brood-mixing by nymphs (Kölliker and Vancassel 2007; Meunier and Kölliker 2012*b*; Wong and Kölliker 2013), and nymph condition was expected to affect nymph incentives to obtain food from siblings.

Material and Methods

Origin of the Tested Individuals

All the clutches used in the following experiments were produced by a total of 125 *Forficula auricularia* females that had been field sampled in Dolcedo, Italy, in May

2009 (experiments 1 and 2) and in September 2012 (experiment 3). Random groups of females and males collected in May 2009 were first maintained in plastic containers (37 cm × 22 cm × 25 cm) to allow mating (Meunier et al. 2012). These containers were kept at 60% humidity, a 14L : 10D photoperiod, and constant 20°C, and individuals received ad lib. artificial food (food composition detailed in Meunier et al. 2012). After 5 months, the females were isolated in petri dishes (diameter 10 cm) for the production of their first clutch of eggs. Because individuals sampled in autumn are generally mated, the females collected in September 2012 were directly isolated in petri dishes for egg production. At isolation, all females were maintained under complete darkness at 15°C and 60% humidity and received artificial food twice a week. These conditions were maintained from egg laying to hatching, except that no food was provided during this period (Kölliker 2007). At hatching, females and their nymphs were transferred to new petri dishes with humid sand as substrate and a plastic shelter as a nest. These families were then maintained in a climate chamber at 20°C, 60% humidity, and a 14L : 10D photoperiod, and they received artificial food every other day (except when the experiments required otherwise; see below) until the end of the experiments. The females sampled in September 2012 were kept under these same conditions (Meunier et al. 2012) until they produced their second clutches, which were used to conduct experiment 3. In *F. auricularia*, females produce up to two clutches in their lifetime (Meunier et al. 2012).

Experiment 1: Do Nymphs Exchange Food, and What Is the Effect of Maternal Presence on This Exchange?

In this first experiment the clutches produced by 45 females were randomly assigned to three experimental treatments, each of them containing 15 replicates (i.e., one clutch per replicate). For all treatments, the replicates consisted of one 4-day-old focal nymph that was food deprived for 1 day before the setup to slightly increase its nutritional needs and marked with a dot of red paint for identification (focal nymph) and three nymphs from the same family fed on green-colored food 1 day before the setup. The green-colored food (mix of blue food dye and yellow pollen) was used as an indirect marker of food sharing (active and/or passive; see experiment 3), because ingested green food is visible through the cuticle of the partially transparent nymphs (fig. A1; figs. A1, A2 available online; Staerkle and Kölliker 2008).

In the first treatment, the groups of nymphs were set up with their mother and could freely interact with her (maternal interaction treatment). In the second treatment, the groups of nymphs were set up with their mother but separated by a mesh to prevent physical contact between nymphs and mothers (maternal presence treatment). Finally, in the third treatment, the nymphs were set up without their mother (maternal absence treatment). One day after the setup of the experimental family groups, we scored whether the focal nymphs were green or not, which is a measurement of whether they received food from their siblings, who were previously fed on green-colored food. The mothers were fed with yellow-colored pollen in the days before the experimental setup to ensure that any green food ingested by the focal nymphs could exclusively originate from sibling nymphs. The nymph's changes into green were determined using a stereomicroscope to record even small amounts of food transfer, and the observer was blind with respect to the experimental treatments. Each group was set up in 50-mm-diameter petri dishes containing humid sand and a metal mesh used either to separate the mother from the nymphs or to simply partition an empty side of the petri dish.

Experiment 2: Can Passive Allo-Coprophagy at Least Partly Mediate Food Transfer among Nymphs?

A split-clutch experiment was conducted using the clutches of 40 females (different from the ones used in experiment 1). Five days after hatching, six nymphs per clutch were assigned to the following three treatments. In the first treatment, one focal nymph was set up in a new petri dish without any food (control, $n = 40$). In the second treatment, one focal nymph was set up in a new petri dish to which we added three nymphs from the same family that had been fed green-colored food during the 2 days before the setup (colored nymphs treatment, $n = 40$). Finally in the last treatment, one focal nymph was set up in a petri dish that contained the frass that had been released by the above three colored nymphs during the 2 days before the setup (colored frass treatment, $n = 40$). Each focal nymph was food deprived for 2 days before the setup to enhance their nutritional need and marked with a dot of red paint. One day after the setup, we determined whether the focal nymphs were green using a stereomicroscope and following a blind procedure (see above). All experimental groups were set up in 15 × 15 × 20-mm plastic boxes with humid sand as substrate.

Experiment 3: (1) Do Allo-Coprophagy, Stomodeal Trophallaxis, and/or Proctodeal Trophallaxis Mediate Food Transfer? (2) What Is the Influence of Nymph-Nymph Relatedness and Nymph Starvation on the Level of Food Transfer?

This third experiment aimed at disentangling (1) whether food transfer between nymphs was mediated by the active

release of frass by the encountered fed nymphs (i.e., socially induced allo-coprophagy), by mouth-to-mouth contacts (which typically mediates stomodeal trophallaxis), and/or by mouth-to-anus contacts (which mediate proctodeal trophallaxis), as well as to test (2) whether the transfer of food between nymphs was shaped by nymph-nymph relatedness and/or nymph starvation. To this end, a full-factorial experiment was conducted using 40 pairs of nymphs that were either related or unrelated and where the focal nymphs were either starved or fed ad lib. (each of the four combinations: $n = 10$). The clutches produced by 40 females were first maintained in their original petri dish for 16 days with ad lib. food, after which nymphs were transferred to a new petri dish, while mothers were discarded to mimic natural family disruption (Meunier et al. 2012). The 40 groups were then maintained in these petri dishes for 10 ± 0.8 (mean ± SE) more days. Three nymphs per group were separated between two new petri dishes. In the first one, two nymphs were set up for 4 days with green-colored food (donor nymphs), whereas in the second one, one focal nymph was isolated (recipient nymph) with either noncolored pollen (fed ad lib.) or without any food source (food deprived). Food deprivation reduced the fresh weight of nymphs by 35.7% at setup (fig. A2; $t = -3.16$, df = 38, $P = .003$) and therefore enhanced nutritional needs in the food-deprived nymphs. After these 4 days, one of the two donor nymphs was randomly paired with the focal recipient nymph, resulting in the four possible combinations of related or unrelated pairs with starved or fed focal nymphs.

At setup, each experimental pair was videotaped for 1 h (Sony Handycam HDR-CX 200) under red light to mimic night conditions (this species is nocturnal). The resulting movies were then analyzed to quantify the number of mouth-to-mouth contacts, the total duration of mouth-to-mouth contacts, the number of mouth-to-anus contacts from the recipient to the donor nymph, and the total duration of these mouth-to-anus contacts. Notice that aggressive behaviors (e.g., the characteristic abdomen lifting and cerci display; Eisner 1960) between nymphs were observed in only three pairs during the experiment (one aggression observed in a related + ad lib. food pair, three in a related + ad lib. food pairs, and 15 aggressions in one unrelated + ad lib. food pair). Video analyses were done using the free software VLC (http://www.videolan.org/) and following a blind observation procedure (see above). All nymphs were 26 days old at setup and emerged from second clutches. We used 26-day-old nymphs as compared to the younger ones used in the first two experiments, because the movies recorded under red-light conditions were of relatively poor quality, thus only allowing fine-scale observations of trophallactic behaviors in larger nymphs. Nymphs of that age are still very keen to aggregate with their mothers (Wong and Kölliker 2012). Because the 26-day-old nymphs are less transparent than the younger ones used in the two first experiments, the amount of food transferred from nonfocal to focal nymphs here was estimated by calculating the weight gained by each focal nymph over the 1 h of filming and not through changes in body coloration. This weight gain was calculated by subtracting the fresh weight of each focal nymph measured to the nearest 1 μg after the experiment (Mikrowaage MYA 5, Pescale) from the one measured before the experiment. After 1 h of filming, each petri dish received a piece of wet paper towel to provide water and humidity. The number of colored frass pellets (i.e., frass released by the donor nymphs) present in the petri dishes was counted after 1 h and 24 h in six replicates per combination (i.e., in a total of 24 pairs). All petri dishes were 50-mm diameter, covered with humid sand.

In 20 out of the 40 families (equally distributed across the four treatments) the mothers were already removed 1 day after hatching. Early presence or absence of tending mothers did not significantly influence any of our measurements (all $P > 0.23$) and its inclusion in the analysis did not qualitatively change any of our conclusions. Thus, this factor was not included in the following statistical analyses.

Statistical Analyses

The results of the first and second experiments were analyzed using a generalized linear model (GLM) and a generalized linear mixed model (GLMM), respectively. In these two models, the coloration of the focal nymphs was entered as binary response variable (with binomial error distributions) and the experimental treatments as explanatory factor. Because each family contributed to the three experimental groups in experiment 2, the GLMM was conducted using family of origin as random effect. Pairwise comparisons between the treatments were conducted using χ^2 tests in which the significance level was Bonferroni adjusted to $\alpha = 0.025$.

The third experiment partly aimed at testing the influence of trophallactic behaviors on the weight gained by focal nymphs. Because we found significant correlations among the mean duration and the number of mouth-to-mouth and mouth-to-anus contacts (see table A1, available online), we first conducted a principal component analysis (PCA) to obtain four noncorrelated principal components (PCs) reflecting single or combinations of trophallactic behaviors (see details in table 1). This PCA was conducted using the $\log(x + 1)$-transformed mean duration and number of mouth-to-mouth and mouth-to-anus contacts between nymphs. The PCs were then used to test whether the weight gained by focal nymphs was mediated by troph-

Table 1: Loadings of the principal component (PC) analysis conducted on the trophallactic behaviors

	PC1	PC2	PC3	PC4
Mouth-to-mouth contacts:				
Mean duration	−.560	−.262	−.566	−.546
Number	−.594	−.418	.131	.674
Mouth-to-anus contacts:				
Mean duration	−.299	.813	−.386	.316
Number	−.494	.308	.717	−.384
Variance explained (%)	47.0	25.9	19.3	7.8

allaxis (i.e., associated with the PCs) and/or by coprophagy (i.e., associated with the frass pellet number). To this end, we conducted a GLM in which nymph weight gain was entered as response variable, while the number of frass pellets counted after 1 h and the four PCs were entered as explanatory variables. We finally tested whether trophallactic behaviors triggered frass release using a GLM in which the $\log(x + 1)$-transformed number of frass pellets counted after 1 h (and then 24 h) was entered as response variable and the four PCs as explanatory variables. These two last models were started by including all possible interactions between the factors and then proceeded with stepwise simplification by removing the interaction terms that were not significant (all $P > .19$).

The influences of relatedness and starvation on the level of food transferred between nymphs in the third experiment were tested using three GLMs in which these factors (and their interaction) were entered as explanatory variables, while the $\log(x + 1)$-transformed number of frass pellets counted after 1 h or 24 h and the weight gained by focal nymphs were separately entered as response variables. Furthermore, the effect of relatedness and starvation (and their interaction) on the trophallactic behaviors was tested using a multivariate analysis of variance (MANOVA) in which the four PCs were entered as response variables. The influence of these fixed factors on each of the PC was then tested using separate GLMs. In all statistical analyses, the $\log(x + 1)$-transformation was used to fulfill residual normality of the models. All statistical analyses were conducted using the software R, version 2.15.2 (http://www.r-project.org/) loaded with the libraries "car" and "Hmisc."

Results

Experiment 1: Do Nymphs Exchange Food, and What Is the Effect of Maternal Presence on This Exchange?

Focal nymphs received food from the nonfocal ones in all three experimental treatments but to variable degree. In the maternal interaction treatment, 33.3% of the focal nymphs became colored, 87% did in the maternal presence treatment, and 66.7% did in the maternal absence treatment (fig. 1*A*; binomial GLM, likelihood ratio [LR] $\chi^2_2 = 9.70$, $P = .008$). The number of colored nymphs was significantly lower in the maternal interaction than the maternal presence treatments ($\chi^2_1 = 8.89$, $P = .003$) but not significantly different between the maternal presence and the maternal absence treatments ($\chi^2_1 = 1.67$, $P = .195$) or between the maternal interaction and the maternal absence treatments ($\chi^2_1 = 3.33$, $P = .068$). Overall, food transfer between nymphs occurred significantly more in the groups where nymphs did not have any possible physical contact with their mothers (76.7% vs. 33.3%, respectively; binomial GLM, $n = 30$, LR $\chi^2_1 = 9.51$, $P = .0021$), but it was not significantly influenced by the presence of mother (whether prevented from physically interacting with the nymphs or not) in the petri dish (60.0% versus 66.7%, respectively; binomial GLM, $n = 30$, LR $\chi^2_1 = 0.19$, $P = .662$).

Experiment 2: Does Allo-Coprophagy at Least Partly Mediate Food Transfer among Nymphs?

Colored focal nymphs were found both in the presence of colored frass and of colored nonfocal nymphs. In particular, 72.5% of the focal nymphs became colored in the colored frass treatment, as did 89.7% in the petri dishes with colored nymphs and 0% in the petri dishes without colored nymphs or frass (fig. 1*B*, binomial GLMM; LR $\chi^2_2 = 28.13$, $P < .0001$). The number of colored nymphs was significantly lower in the control than in both the colored frass ($\chi^2_1 = 45.49$, $P < .0001$) and the colored nymph ($\chi^2_1 = 67.84$, $P < .0001$) treatments. Furthermore, a significantly larger proportion of nymphs became colored in the petri dishes with colored nymphs than in the ones with colored frass ($\chi^2_1 = 5.31$, $P = .021$), indicating that other factors than allo-coprophagy at least partly mediate food transfer among nymphs.

Experiment 3: (1) Do Allo-Coprophagy, Stomodeal Trophallaxis, and/or Proctodeal Trophallaxis Mediate Food Transfer?

The PCA conducted on the mean duration and number of mouth-to-mouth and mouth-to-anus contacts between nymphs provided four orthogonal PCs (table 1). All four traits exhibited consistent negative loadings on PC1, indicating a (low) general time investment in trophallactic behaviors. In other words, high values for PC1 reveal overall low frequencies and low durations for the trophallactic behaviors. Conversely, the mean duration of mouth-to-anus contacts primarily and positively loaded on PC2,

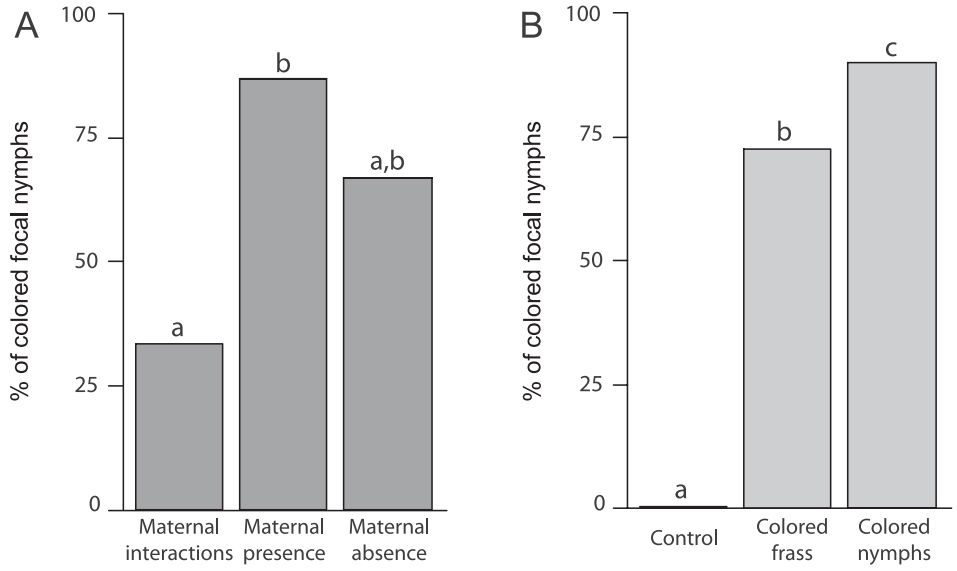

Figure 1: Percentage of experimental groups where the focal nymph became colored after being in contact with colored nymphs and in contact with their mothers (maternal interactions), near their mothers but not in contact with them (maternal presence), or without any mother (maternal absence) (*A*); and either alone (control), in contact with colored frass, or in contact with colored nymphs (*B*). Different letters indicate significant differences between the treatments (see text).

which thus reflects the duration of proctodeal trophallactic behaviors. The number of mouth-to-anus contacts primarily and positively loaded on PC3, which indicates the number of proctodeal trophallactic behaviors. Finally, the number and duration of mouth-to-mouth contacts strongly loaded on PC4 (but in the opposite direction), thus reflecting a trade-off between number and duration of stomodeal trophallactic behaviors.

The weight gained by the recipient nymphs in experiment 3 was significantly and positively associated with the number of frass pellets released by the donor nymph counted after 1 h (fig. 2*A*; GLM; LR $\chi_1^2 = 7.77$, $P = .005$), as well as to PC2 (fig. 2*B*; GLM; LR $\chi_1^2 = 5.37$, $P = .020$). In other words, recipient nymphs gained more weight when they had access to more frass but also when they exhibited longer mouth-to-anus contacts (i.e., proctodeal trophallactic behaviors). By contrast, the weight gained by focal nymphs was not significantly influenced by PC1 (LR $\chi_1^2 = 2.35$, $P = .130$), by PC3 (LR $\chi_1^2 = 0.10$, $P = .756$), by PC4 (LR $\chi_1^2 = 2.37$, $P = .123$), or by any interactions among them (all $P > .19$). Trophallactic behaviors did not trigger frass release, as revealed by the fact that the number of frass pellets counted after 1 h was nonsignificantly influenced by PC2 (LR $\chi_1^2 = 2.23$, $P = .135$) and any other PCs (PC1: LR $\chi_1^2 = 3.36$, $P = .07$; PC3: LR $\chi_1^2 = 1.03$, $P = .310$; PC4: LR $\chi_1^2 = 1.76$, $P = .184$).

Experiment 3: (2) What Is the Influence of Nymph-Nymph Relatedness and Nymph Starvation on the Level of Food Transfer?

As predicted, if frass production is a (socially induced) kin-directed process, we found that donor nymphs produced twice as much frass after 24 h when in contact with a related nymph than with an unrelated nymph (fig. 3*A*; LR $\chi_1^2 = 7.46$, $P = .006$). Notice that relatedness did not significantly influence the number of frass pellets counted in the short term (i.e., 1 h; LR $\chi_1^2 = 2.13$, $P = .145$), even if the trend followed the same direction as the significant one observed after 24 h (fig. 3*B*). The total number of frass pellets counted after 24 h and 1 h, respectively, was not significantly influenced by starvation (24 h: LR $\chi_1^2 = 0.001$, $P = .975$; 1 h: LR $\chi_1^2 = 0.85$, $P = .357$) or by an interaction between food deprivation and relatedness (24 h: LR $\chi_1^2 = 1.81$, $P = .179$; 1 h: LR $\chi_1^2 = 0.57$, $P = .450$).

In addition to an effect of relatedness on frass release, we found an overall effect of relatedness on the trophallactic behaviors (MANOVA on the four PCs; approximated $F_{4,33} = 3.27$, $P = .023$). This overall effect was mainly driven by PC2, which was significantly larger in nonrelated than related pairs (table 2; fig. 3*C*), thus indicating that the durations of mouth-to-anus contacts were longer between unrelated than related individuals. None of the other

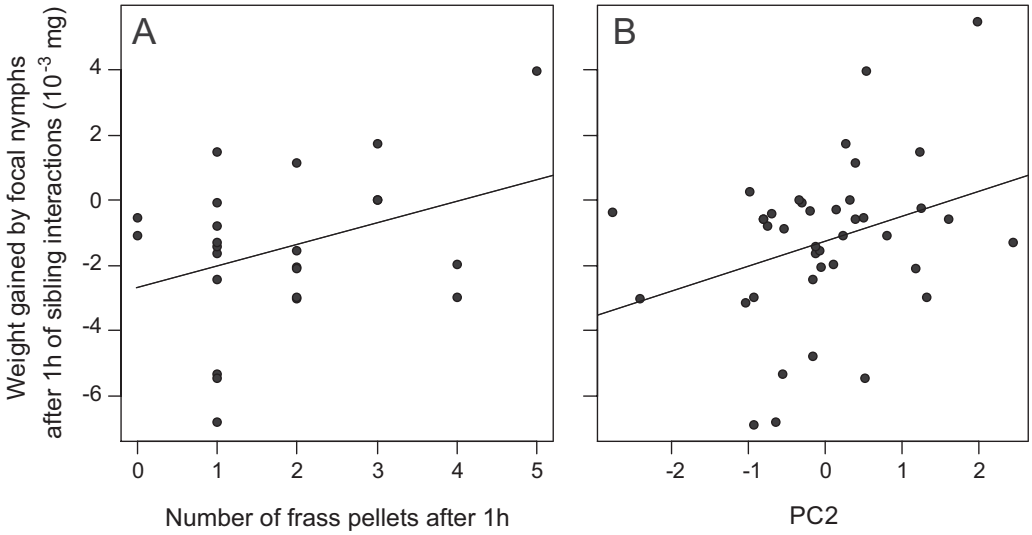

Figure 2: Positive association between the weight gained by focal nymphs and the number of frass pellets released by the nonfocal nymphs over 1 h (*A*) and PC2, which mainly reflects the mean duration of mouth-to-anus contacts (*B*).

PCs was significantly influenced by relatedness, starvation, or an interaction between these two factors (table 2). Finally, the trophallactic behaviors overall were not significantly influenced by starvation (approximately $F_{4,33}$ = 0.50, P = .735) or by an interaction between starvation and relatedness (approximately $F_{4,33}$ = 0.50, P = .739). Interestingly, the weight gained by the recipient nymphs over 1 h was not significantly influenced by relatedness (LR χ^2_1 = 1.87, P = .172), starvation (LR χ^2_1 = 0.06, P = .805), or an interaction between relatedness and starvation (LR χ^2_1 = 0.29, P = .591).

Discussion

Our study demonstrates that sibling cooperation through food sharing does occur among nymphs of same age in families of the European earwig. Specifically, we showed that (1) the food ingested by individual offspring is frequently transferred to siblings during family life and that (2) active allo-coprophagy and proctodeal trophallaxis mediated such food exchange. Interestingly, our results also revealed that (3) relatedness shaped allo-coprophagy and proctodeal trophallaxis in opposite directions: offspring interacting with a related nymph produced significantly more frass (thus favoring food transfer through allo-coprophagy), whereas offspring interacting with an unrelated nymph exhibited longer mouth-to-anus contacts (thus favoring food transfer through proctodeal trophallaxis). This effect of relatedness on the two mediators of food transfer between nymphs (4) did not translate into an overall effect of relatedness on the total amount of food transferred to the recipient nymphs. Hence, relatedness influenced the strategy used by nymphs to provide and/or obtain food from other nestmates, but the two outcomes resulted in equivalent outcomes in terms of amount of food transferred (at least over the course of the experiment). Finally, (5) physical interactions with the mother reduced, albeit did not prevent, the likelihood of food transfers between nymphs and (6) food-deprived nymphs did not receive more food from their siblings than nondeprived ones.

The results that nymphs exchanged food through active allo-coprophagy and proctodeal trophallaxis and that such exchanges occurred mainly without aggressive behaviors overall support the hypothesis that food sharing reflects an active form of cooperation rather than an opportunistic and passive feeding on waste produced by other individuals. Sibling food sharing is a well-known and an obligate behavior in cooperative breeders and eusocial insect societies (e.g., ants, bees, wasps and termites) where the development and survival of newly produced offspring rely on sibling care and food provisioning. Care for juveniles is in most of these cases provided by adult siblings (i.e., workers, helpers) that stay in the family group after reaching adulthood (Wilson 1971; Hatchwell and Komdeur 2000; Cant 2012), although it was recently shown to also occur among juvenile siblings (larvae) in ambrosia beetles (*Xyleborinus saxesenii*; Biedermann and Taborsky 2011). However, sibling food sharing has been rarely studied and described in more primitive social groups, such as the ones consisting of facultative caring parents, where sibling interactions are limited to contemporary offspring from the same brood. By demonstrating that active sibling food

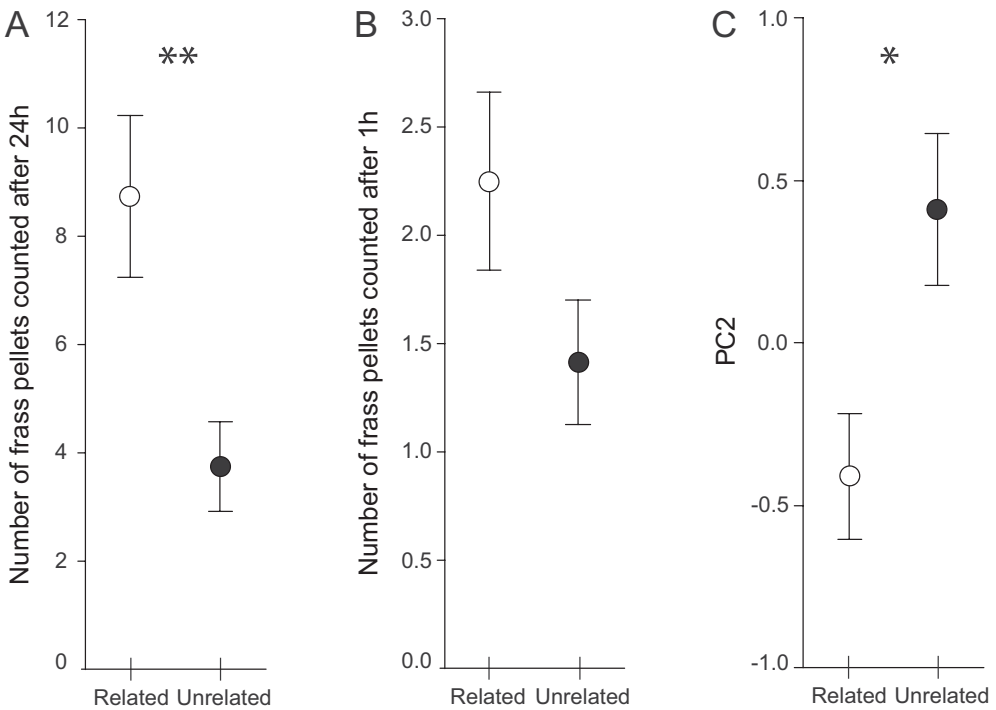

Figure 3: Influence of relatedness between focal and nonfocal nymphs on the number of frass pellets produced by the nonfocal nymphs after 24 h (*A*), on the number of frass pellet produced by the nonfocal nymphs after 1 h (*B*), and finally on PC2, a principal component positively associated to the mean duration of mouth-to-anus contacts between nymphs (*C*). All values are mean ± SE. One asterisk, $P < .05$; two asterisks, $P < .01$.

sharing occurs in such a species, our study provides evidence that sibling cooperation in the form of food sharing may not be a derived trait requiring a cooperative breeding or eusocial system. Instead, we suggest that sibling food sharing is an ancestral behavior that promoted the early evolution of social life. In particular, cooperation between contemporary siblings may increase the incentive of mobile offspring to stay and aggregate with their siblings, which in turn facilitates the evolution of parental care and, ultimately, of more derived forms of social life.

We showed that nymphs received nutritional benefits from sibling food sharing in terms of weight gain, but the experimental reduction of weight in recipient nymphs did not trigger higher rate of food transfer. This finding reveals that food deprivation is not the main driver of sibling food sharing in earwigs and suggests benefits additional to the ones reflected purely by food intake. In line with this hypothesis, our experiment demonstrated that food transfer was mediated by allo-coprophagy and proctodeal trophallaxis, two behaviors typically associated with three benefits for the recipient (reviewed in Nalepa et al. 2001; Weiss 2006). First, allo-coprophagy allows the digestion of microbes that quickly colonize frass pellets after gut transit, a mechanism known to provide an important and sometimes the unique source of protein, lipid, carbohydrates, or micronutrients to the recipient (Martin and Reddy 1984). Second, allo-coprophagy and proctodeal trophallaxis also permit specific food sources to be preprocessed by microbes or individuals, respectively, thereby facilitating their otherwise difficult assimilation in the recipient organism. This mechanism (called external rumen; Nalepa et al. 2001) is known to enhance the digestion of cellulose, allow the detoxification of allelochemicals, and/or soften the food in several insect and mammal species (Nalepa et al. 2001). Finally, allo-coprophagy and proctodeal trophallaxis allow individuals to acquire the microbes present in the ingested frass to establish a mutualistic hindgut fauna. Such a process is considered as a key step in the evolutionary transition from the more primitively social cockroaches to the complex eusocial termite colonies in which wood digestion requires the transfer of microbial fauna from mothers to offspring and/or between siblings (Nalepa and Bell 1997; Nalepa et al. 2001; Pellens et al. 2007; Bignell 2011). In *Forficula auricularia*, the benefits of digesting microbes (i.e., the first benefits described above) are unlikely to drive sibling food sharing, as in-

Table 2: Influence of relatedness and starvation on the four principal components (PCs) obtained from principal component analysis on the trophallactic behaviors

	PC1		PC2		PC3		PC4	
	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*
Relatedness	3.23	.072	7.09	**.008**	.35	.552	1.13	.287
Starvation	.22	.640	.25	.614	1.57	.210	.10	.753
Relatedness : starvation	.27	.603	.02	.891	.43	.513	1.41	.235

Note: The significant effect is in bold. LR = likelihood ratio.

dividuals are mobile and omnivorous and thus likely to have direct access to all the types of nutrients required for their development and survival (Albouy and Caussanel 1990). Whether earwig nymphs consume frass to facilitate the assimilation of certain types of food and/or to establish a mutualistic hindgut fauna will have to be investigated in further studies.

Relatedness is recognized as a keystone in the evolution of cooperation and sociality, because the indirect fitness benefits gained by helping relatives possibly outweigh the direct costs of cooperation (Hamilton 1964; West et al. 2002). Surprisingly, our study shows that relatedness did not influence the total amount of food exchanged between nymphs, even though interactions among unrelated nymphs frequently occurs under natural conditions due to brood mixing (Kölliker and Vancassel 2007; Wong and Kölliker 2013). Instead, relatedness had more subtle effects on food sharing by affecting the behavioral mechanism by which individuals provided and/or obtained food from their nestmates. In particular, high relatedness increased frass release (which in turn favored food transfer through allo-coprophagy), whereas low relatedness increased the duration of mouth-to-anus contacts (which favored food transfer through proctodeal trophallaxis). The increase in frass release among related individuals is likely to be an effect on donor generosity and resembles the kin-directed production of a public good (e.g., in bacteria; Griffin et al. 2004; Diggle et al. 2007) that could benefit all nymphs within a family. Conversely, it is unclear whether longer proctodeal trophallaxis between unrelated individuals reflects a cooperative behavior of the donor (which increased frass supply), or a competitive behavior of the recipient nymph (which increased its demand and monopolized frass early). The use of proctodeal trophallaxis instead of allo-coprophagy to mediate food transfer could thus reveal a behavioral conflict between unrelated nymphs, a scenario in line with the higher rates of cannibalism reported between unrelated as compared to related earwig nymphs (Dobler and Kölliker 2011).

Physical contacts between mothers and offspring are known to include a range of behaviors in European earwigs, such has grooming, aggression, antennations, and the transfer of food from mothers to offspring (Staerkle and Kölliker 2008; Mas and Kölliker 2011). Here we showed that direct physical contacts between mothers and offspring limited the frequency of sibling food sharing. This result suggests that offspring prefer the maternally provided food to the sibling-provided food, possibly because the former is transferred in larger quantities or has a higher quality. Nevertheless, sibling food sharing still occurred in 33% of the experimental groups tended by a mother. The level of maternal care and food provisioning are known to vary broadly among *F. auricularia* mothers, ranging from females exhibiting high provisioning rates to females provisioning none of their nymphs (Mas and Kölliker 2011; Meunier et al. 2012; Meunier and Kölliker 2012*a*). The occurrence of sibling food sharing observed in the maternal interaction treatments could thus reflect a context-dependent strategy of earwig nymphs to ensure the gain of food already ingested by another individual (and its possibly associated benefits): nymphs obtain food from siblings when the mother is absent but maybe also when the mother shows a low level of provisioning.

To conclude, our study demonstrates that sibling interactions during *F. auricularia* family life include cooperative behaviors in the form of food sharing. Together with previous studies showing the occurrence of siblicide and cannibalism between earwig nymphs (Dobler and Kölliker 2010, 2011), our results reveal the broad spectrum of behaviors from antagonistic to cooperative characterizing sibling interactions in this species. Because *F. auricularia* is a species that exhibits facultative forms of maternal care (Kölliker 2007) probably reflecting an early intermediate level in the transition from solitary to highly developed forms of social life, our findings also provide evidence that the evolutionary forces promoting the emergence and the persistence of parental care and social life do not (necessarily) rely only on the benefits of parental care for offspring but may also involve the benefits of cooperative sibling interactions—possibly even before the evolution of parental care. These results claim for more considerations in further theoretical and empirical studies investigating the role of sibling cooperation in the early evolution of parental care and social life.

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Left, an earwig mother (*Forficula auricularia*) tending her clutch of young offspring. *Right*, siblings interact in absence of their tending mother. Photo credit: Joël Meunier.

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Negative association between parental care and sibling cooperation in earwigs: a new perspective on the early evolution of family life?

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Keywords:

Forficula auricularia;
insects;
maternal care;
precocial species;
sibling rivalry;
social evolution.

Abstract

The evolution of family life requires net fitness benefits for offspring, which are commonly assumed to mainly derive from parental care. However, an additional source of benefits for offspring is often overlooked: cooperative interactions among juvenile siblings. In this study, we examined how sibling cooperation and parental care could jointly contribute to the early evolution of family life. Specifically, we tested whether the level of food transferred among siblings (sibling cooperation) in the European earwig *Forficula auricularia* (1) depends on the level of maternal food provisioning (parental care) and (2) is translated into offspring survival, as well as female investment into future reproduction. We show that higher levels of sibling food transfer were associated with lower levels of maternal food provisioning, possibly reflecting a compensatory relationship between sibling cooperation and maternal care. Furthermore, the level of sibling food transfer did not influence offspring survival, but was associated with negative effects on the production of the second and terminal clutch by the tending mothers. These findings indicate that sibling cooperation could mitigate the detrimental effects on offspring survival that result from being tended by low-quality mothers. More generally, they are in line with the hypothesis that sibling cooperation is an ancestral behaviour that can be retained to compensate for insufficient levels of parental investment.

Introduction

The evolution of social life requires that the benefits individuals gain through group living outweigh its inherent costs (Alexander, 1974; Bourke, 2011). These costs typically arise from a higher risk of pathogen transmission (Schmid-Hempel, 1998; Altizer *et al.*, 2003), as well as from an increased intensity of competition for limited resources and reproduction (Mock & Parker, 1997; Krause & Ruxton, 2002; Roulin & Dreiss, 2012). Conversely, the benefits of social life are usually attributed to social interactions among group members that can, for example, enhance predator defence and foraging efficiency (Krause & Ruxton, 2002; Royle *et al.*, 2012). The basic challenge in understanding the evolution of social life is thus to unravel the nature and functional interactions of mechanisms underlying the net benefits of group living (Alexander, 1974; Bourke, 2011).

Our current knowledge of the mechanisms that shape social evolution mostly stems from studies on the highly derived social systems of mammals, birds and eusocial insects (e.g. Wilson, 1971; Royle *et al.*, 2012), which are characterized by obligatory and often permanent forms of group living. However, only little attention has been paid to the study of less derived stages of social evolution, such as those found in species exhibiting facultative and/or temporary forms of family life with parental care. Investigating the interplay of evolutionary mechanisms that underlie the net benefits of group living in such species is crucial to expand our understanding of the emergence of social life from an ancestral, solitary state (Smiseth *et al.*,

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2003; Falk *et al.*, 2014; Meunier, 2015), which is considered to be one of the major transitions in the evolution of life (Szathmáry & Maynard Smith, 1995; Bourke, 2011).

Over the last decades, offspring benefits of family life were traditionally attributed to interactions between parents and offspring in the form of parental care (reviewed in Royle *et al.*, 2012). However, an often overlooked source of benefits is sibling cooperation (Forbes, 2007; Roulin & Dreiss, 2012), which is promoted by the additional indirect fitness benefits of assisting genetically related individuals (Hamilton, 1964; West *et al.*, 2002). Cooperation among adult siblings is common in nature, such as in cooperatively breeding vertebrates (Clutton-Brock, 2002; Koenig & Dickinson, 2004) or in colonies of eusocial insects (Wilson, 1971), but an increasing number of studies also report cooperation among juvenile siblings. For instance, offspring express mutual cleaning in the Mississippi kite *Ictinia mississippiensis* ('allo-preening'; Botelho *et al.*, 1993) and the ambrosia beetle *Xyleborinus saxesenii* (Biedermann & Taborsky, 2011), as well as share food in the Common barn owl *Tyto alba* (Roulin *et al.*, 2012), the huntsman spider *Delena cancerides* (Yip & Rayor, 2013) and the European earwig *Forficula auricularia* (Falk *et al.*, 2014).

Although both sibling cooperation and parental care may provide substantial benefits to juveniles during family life, it remains surprisingly unexplored how these behaviours are related when they co-occur. Assessing the modality of their co-occurrence would allow to determine their independent or joined roles, as well as their respective importance in the evolutionary transition from solitary to group living (Falk *et al.*, 2014). The association between sibling cooperation and parental care – if any – could either be complementary or compensatory. In the first case (here termed complementarity hypothesis), the level of sibling cooperation is predicted to be positively correlated with the level of parental care. This scenario could, for example, be based on a higher propensity of siblings to cooperate with each other when the level of parental care is high, which in turn should reduce offspring competition and conflict (Roulin & Dreiss, 2012) that are otherwise predicted to hamper cooperation (Frank, 1998). Such a positive correlation could be expected in altricial species, in which offspring exclusively rely on parental resources. In the second case (here termed compensation hypothesis), the level of sibling cooperation is expected to be negatively associated with the level of parental care. This scenario likely applies to precocial species, which exhibit a nonderived and nonobligatory form of family life. In these species, offspring do not exclusively rely on parental resources, but instead either have direct access to the resources used as nest material (e.g. carrion and dung) or are mobile and capable of independent resource acquisition in the vicinity of the nest site. Consequently, offspring competition over parental resources could be reduced and offspring could benefit from sharing independently acquired resources with siblings (Falk *et al.*, 2014), particularly when parental investment is insufficient. Under such circumstances, resource transfer among siblings could even release parents (at least partly) from offspring provisioning.

In this study, we examined whether food transfer among siblings (a form of sibling cooperation) and food provisioning by parents (a form of parental care) are complementary, compensatory or independent behaviours in the European earwig *Forficula auricularia* L. In this precocial insect species, mothers care for their mobile offspring (called nymphs) for several weeks after hatching (Lamb, 1976a). During this period, maternal care includes the protection and grooming of nymphs as well as their provisioning with food through regurgitation (Lamb, 1976b; Staerkle & Kölliker, 2008). However, maternal presence and post-hatching care are not obligatory for offspring survival (Lamb, 1976b; Kölliker, 2007), as nymphs do not only acquire food through maternal provisioning, but forage independently soon after hatching (Lamb, 1976b; Wong & Kölliker, 2012) and share food with their siblings (Falk *et al.*, 2014). Within earwig families, this food transfer among siblings has been shown to be predominantly mediated by active allo-coprophagy, a form of sibling cooperation defined by a socially induced increase in faeces production by donor nymphs and the subsequent consumption of these faeces by recipient siblings (Falk *et al.*, 2014).

To unravel the relation between parental care and sibling cooperation in earwig families, we measured the co-expression of maternal food provisioning and sibling food transfer. Because group size can be an important parameter in family interactions that is classically assumed to affect the intensity of competition among group members (Alexander, 1974; Shen *et al.*, 2014) and has been linked to differences in mortality and developmental rates in European earwigs (Kölliker, 2007; Meunier & Kölliker, 2012b), we first tested (1) whether group size (offspring number) shapes the expression of sibling food sharing and maternal food provisioning. We then investigated (2) the nature and direction of the potential association between the two types of food transfer. Finally, we tested whether the level of sibling food transfer (3) affects offspring fitness and/or (4) reflects the quality of the tending mothers. To these ends, we first investigated whether variations in the level of food transfer were associated with changes in offspring development and survival, and then with the number of eggs produced by mothers in their following (and final) reproductive attempt.

Materials and methods

Study animals and laboratory rearing

The adult female earwigs used in our experiment descended from 160 individuals collected in a natural population in Dolcedo, Italy, in September 2012. These field-caught earwigs had been maintained in the laboratory under standard rearing conditions (detailed in Meunier *et al.*, 2012; Koch & Meunier, 2014) for one generation. After emergence, F1 adults were maintained in large plastic containers (37 × 22 × 25 cm) for 3 months to allow uncontrolled mating in sex-balanced groups of adults with different genetic origins. The following experiment involved a random sample of 54 of these F1 females and their subsequent first clutch of offspring.

General experimental set-up

We successively measured the levels of sibling food transfer and maternal food provisioning in 54 clutches (see experimental details in Fig. 1). Four days after egg hatching, mothers were isolated from their clutches and the nymphs randomly attributed to either of two groups of equal size termed SFT and MFP group, respectively. The SFT groups were used to measure the level of sibling food transfer (details below), whereas the MFP groups were used to measure the level of maternal food provisioning (details below). Because the mothers were involved in both types of measurements, 28 clutches were used to first measure sibling food transfer (days four to five) and then maternal food provisioning (days eight to nine), whereas the order of tests was reversed in the other 26 clutches (Fig. 1). In between the two measurements (i.e. from day five to day eight), the two groups of nymphs were maintained separately and received an *ad libitum* amount of uncoloured pollen pellets as food source (naturally yellow-coloured flower pollen formed into pellets; Hochland Bio-Blütenpollen, Hoyer GmbH, Polling, Germany). During the same period, mothers were isolated and likewise fed with uncoloured pollen pellets.

Once sibling food transfer and maternal food provisioning had been measured, we investigated the association between these measurements and offspring fitness and/or female quality by measuring nymph development and survival, as well as maternal investment in their 2nd clutch. To this end, nymphs from SFT and MFP groups were reassembled with their mother on day nine. Five days later, the mothers were isolated to mimic natural family disruption and allow the production of a second clutch (Meunier *et al.*, 2012), whereas the nymphs were maintained in groups until adult emergence. Nymph development time was recorded by checking daily for the emergence of the first second-instar nymph in each clutch, and the proportion of nymphs that survived until adulthood was assessed by counting the number of nymphs that moulted into adults and then dividing this number by the number of nymphs that initially entered the experiment. Finally, maternal investment in their 2nd clutch – if any – was measured by counting (1) the number of days between their isolation for 2nd clutch production and oviposition, (2) the number of 2nd clutch eggs present 3 days after the first egg laying had been observed (egg laying takes up to 3 days), as well as (3) the number of 2nd clutch nymphs present 1 day after the first hatching had been observed (egg hatching generally occurs over a single day).

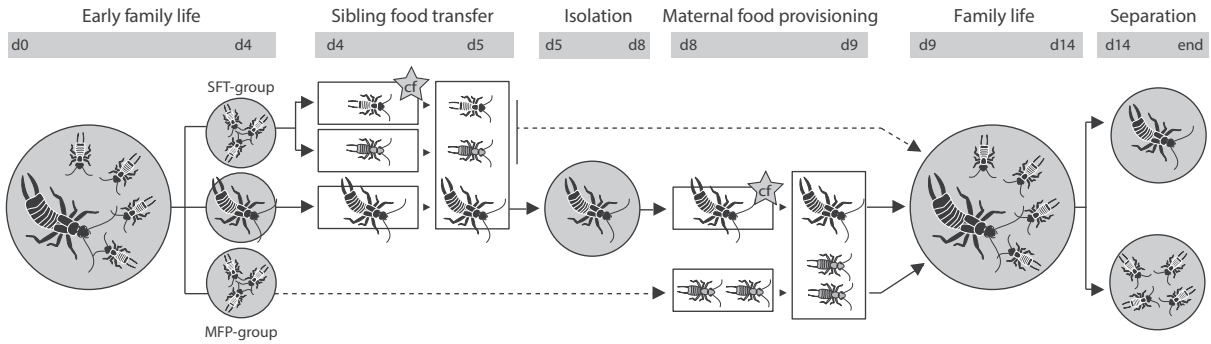

Fig. 1 Experimental set-up. White boxes indicate measurements of sibling food transfer and maternal food provisioning. Sibling food transfer was measured by providing half of the nymphs of the SFT group (called donor nymphs) with coloured food (indicated with 'cf'), then reassembling these newly coloured nymphs with their remaining, food-deprived siblings (called recipient nymphs, grey individuals) and their mother, allowing family interactions overnight and finally counting the number of recipient nymphs that ingested the coloured food provided by the donor nymphs. Conversely, maternal food provisioning was measured by providing the mother with coloured food, then reassembling the fed mother with the MFP group of nymphs previously set aside, allowing them to interact overnight and finally counting the number of nymphs that ingested the coloured food provided by the mother. Note that the order of food transfer test was reversed in half of the tested families.

Groups of nymphs (and, when not isolated separately, their mother) were maintained in medium-sized Petri dishes (9 × 2 cm) until the end of family life (on day 14) and subsequently in large Petri dishes (13.8 × 2 cm) until adult emergence. Each Petri dish contained humid sand as ground material and a plastic tube as shelter. During their isolation from day five to eight, mothers were maintained in small Petri dishes (5.5 × 1.2 cm) inlaid with a moist paper towel. Mothers and nymphs received *ad libitum* uncoloured pollen pellets as food source from hatching to day three. Conversely, nymph and mothers were provided with an *ad libitum* amount of artificial diet twice a week from day nine until the end of the experiment (food composition detailed in the supplementary material). Note that we used pollen instead of this artificial diet during sibling food transfer and maternal food provisioning tests because of its better dyeability. Groups of nymphs and mothers were always food-deprived 1 day prior to the sibling food transfer and maternal food provisioning tests to increase foraging and solicitation behaviours (of the nymphs) on the following day (Staerkle & Kölliker, 2008; Falk *et al.*, 2014).

Measuring sibling food transfer and maternal food provisioning

The measurements of sibling food transfer and maternal food provisioning were implemented by taking advantage of an exceptional property of *F. auricularia* nymphs: ingested coloured food is visible through the partially transparent cuticle of first-instar nymphs and can thus be used as a marker of food transfer between family members (Staerkle & Kölliker, 2008; Falk *et al.*, 2014). In brief, sibling food transfer was measured by (1) providing half of the nymphs of the SFT group with green-coloured food, then (2) reassembling these coloured (donor) nymphs with their uncoloured remaining siblings (recipients) and their mother, (3) allowing family interactions overnight and finally (4) counting the number of newly coloured recipient nymphs. To this end, we first divided each SFT group into two subgroups of equal size (Fig. 1). All nymphs of one of the subgroups were marked by clipping off the distal third of the right cercus (Wong & Kölliker, 2013). This marking had no influence on the proportion of newly green-coloured nymphs in the sibling food transfer tests (Wilcoxon rank sum test; $W = 288.5$, $P = 0.967$). After marking, we randomly selected either the marked or the unmarked subgroup and transferred it for one hour to a small Petri dish containing an *ad libitum* amount of green-coloured food (donor subgroup; naturally yellow-coloured pollen mixed with a blue food dye; Dekoback, Online Ideen GmbH, Germany). Meanwhile, the other (recipient) subgroup was food-deprived, whereas the mother was provided separately with uncoloured pollen. The nymphs of the donor and recipient subgroup were then assembled overnight with their mother in a medium-sized Petri dish. Fifteen hours after the set-up, we counted all nymphs in their respective subgroups and determined the number of newly green-coloured nymphs in the recipient subgroup under a stereomicroscope. We fed mothers, and thus allowed for maternal provisioning during sibling food transfer tests, because this ensured a more direct link between our measures of maternal food provisioning and sibling food transfer. Note that mothers were isolated and fed with uncoloured pollen pellets between day five and eight to ensure that the faeces of mothers first involved in the maternal food provisioning measurement had lost their coloration before sibling food transfer measurements.

We refrained from feeding a fixed number of donor nymphs across clutches of different size, because this would have artificially increased competition for coloured faeces in large clutches. Instead, always feeding half of the nymphs of the SFT group ensured that the *per capita* availability of faeces for recipient nymphs was *a priori* independent of clutch size. Because the higher absolute amount of faeces available during sibling food transfer tests in larger clutches could potentially promote competition among multiple recipients and thus influence the distribution of faeces (see also discussion), we additionally assessed the intensity of coloration in a random subset of 31 clutches by differentiating between strongly and weakly coloured nymphs. Strongly coloured nymphs generally exhibit a homogenous coloration of their entire body that is visible to the naked eye, whereas weakly coloured nymphs only show a light coloration of their gut that can often only be seen on their ventral side and when using binoculars. If competition for a constant *per capita* amount of faeces increased with clutch size, we would expect an increase in sibling food transfer with clutch size accompanied by an increase in the proportion of nymphs that received only little food from their siblings and thus were only weakly coloured.

Maternal food provisioning was measured by (1) providing the mother with green-coloured food, then (2) reassembling the fed mother with the nymphs of the MFP group, (3) allowing them to interact overnight and finally (4) counting the number of nymphs that ingested the coloured food provided by the mother. Specifically, maternal food provisioning was measured using the entire MFP group, in which half of the nymphs were marked by clipping their cercus to ensure that marking could not hamper comparisons between maternal food provisioning and sibling food transfer. Marking the nymphs did not affect maternal provisioning (Wilcoxon signed rank test; $V = 191.5$, $P = 0.962$). After the nymphs had been marked, the mother had access to coloured food for one hour, whereas all the nymphs were food-deprived. Subsequently, the nymphs of the MFP group and their mother were assembled

overnight in a medium-sized Petri dish to allow food transfer between individuals. Note that the number of recipient nymphs during the maternal food provisioning test was large (i.e. twice the number of recipient nymphs used in the sibling food transfer test) to account for the higher absolute amount of food that mothers can potentially provide to their offspring (Mas & Kölliker, 2011; Meunier & Kölliker, 2012a; Meunier *et al.*, 2012). Fifteen hours after the set-up, we counted the number of marked and unmarked green-coloured nymphs. Overall, the scoring of nymphal coloration was performed by one single observer and – in case of the second food transfer test – blindly regarding the level of food transfer measured during the first food transfer test in the same clutch.

Statistical analyses

All statistical analyses were performed using the software R 3.0.1 (http://www.r-project.org/) complemented with the packages 'car' and 'MASS'. We first tested the overall importance of sibling food transfer (SFT) and maternal food provisioning (MFP) on the gain in coloration of recipient nymphs using a generalized linear mixed model (GLMM) with a binomial error distribution corrected for overdispersion. In this model, the proportion of coloured recipient nymphs was entered as response variable (via the 'cbind' function in R), whereas the type of test (SFT or MFP; categorical), the order of the tests (SFT/MFP or MFP/SFT; categorical), clutch size (continuous) and all interactions among these three factors were entered as explanatory variables. Because each clutch was used to measure both sibling food transfer and maternal food provisioning, clutch ID was entered as a random factor into the model. To control whether differences in the number of recipient nymphs involved in the sibling food transfer and maternal food provisioning tests drove the results of the above model, we conducted an additional linear mixed model (LMM), in which we used the same set of explanatory and random variables but entered the absolute number of coloured recipient nymphs as response variable. The potential influence of clutch size on the distribution of food among multiple recipient nymphs during sibling food transfer tests was analysed in a generalized linear model (GLM) with binomial error distribution corrected for overdispersion. In this model, we entered the proportion of weakly coloured nymphs among all coloured recipients as response and clutch size (continuous) as explanatory variable.

We then analysed whether the level of sibling food transfer was positively (complementarity hypothesis) or negatively (compensation hypothesis) associated with the level of maternal food provisioning in each clutch. To this end, we tested the correlation between the deviations from the predicted levels of sibling food transfer and of maternal food provisioning using a Pearson product–moment correlation. These deviations were defined as the residuals of sibling food transfer and maternal food provisioning from the first model, that is the parts of the proportions of coloured recipient nymphs in sibling food transfer and maternal food provisioning tests, respectively, that were not explained by clutch size and order of testing. Note that we back-transformed the residuals to their original (i.e. proportional) scale to facilitate their interpretation in the figures.

Finally, we tested whether deviations from the predicted level of sibling food transfer (see above for definition) were linked to offspring fitness and/or maternal investment in the 2nd clutch. We calculated a series of four linear models and three generalized linear models with binomial error structure corrected for overdispersion. In the linear models (LMs), the deviations from sibling food transfer were entered as an explanatory variable, and the development time of nymphs, the time from isolation to 2nd clutch production, the number of eggs or the number of nymphs in the 2nd clutch as continuous responses. In the generalized linear models (GLMs), we entered the proportion of 1st clutch nymphs that survived until adulthood (continuous), the occurrence of 2nd clutch production (bimodal) or the hatching success of the 2nd clutch (continuous) as response variable. Note that we also tested the effect of deviations from the predicted level of maternal food provisioning on the above-mentioned measures of offspring fitness and maternal investment in the 2nd clutch. The results of the corresponding analyses, which resemble the results based on the deviation from the predicted level of sibling food transfer but in opposite directions, are given in Table S1.

Statistical models were simplified stepwise by removing nonsignificant interaction terms ($P > 0.05$). To correct for multiple testing, the significance level for the analyses of maternal 2nd clutch production and fitness traits in offspring was adjusted using the MFDR (mean false discovery rate) approach to $\alpha c = 0.029$ according to $\alpha c = (n + 1)/(n \times 2) \times 0.05$, where n denotes the number of tests. Our analyses involved 48 of the 54 clutches initially set-up. Among the 6 clutches not used in the analyses, (1) three were excluded because nymphs of the donor group either escaped or were cannibalized by their siblings, which potentially could have biased our measure of food transfer, (2) two clutches showed an exceptionally high proportion of donor nymphs that failed to feed on the green-coloured food prior to the sibling food transfer test (50% and 70%, respectively, vs. 4 ± 8% (mean ± SD) in the remaining clutches), and finally (3) one clutch was excluded because the mother still produced green-coloured faeces prior to the sibling food transfer test, which prevented reliable measurements of sibling food transfer. As a result, the analysed data set comprised 24 of the 28 clutches that were subjected to sibling food transfer measurements on day four, and 24 of the 26 clutches

used to measure sibling food transfer on day eight. A total of 41 of 48 (85.4%) mothers produced a 2nd clutch and were used to analyse the time from isolation to 2nd clutch production, as well as the hatching success of the 2nd clutch.

Results

Overall, food transfer among nymphs occurred more frequently than maternal provisioning (94% vs. 67% of all clutches; $\chi_1^2 = 9.45$, $P = 0.002$). The proportion of newly coloured recipient nymphs varied substantially between families and ranged from 0 to 100% after both types of food transfer tests, with a median value of 75% of recipient nymphs newly coloured in the sibling food transfer test and 22% in the maternal food provisioning tests (Wilcoxon signed rank test, $V_{47} = 169$, $P < 0.001$; Fig. S1). These values are comparable to the proportions presented in previous studies (Meunier & Kölliker, 2012a; Meunier *et al.*, 2012; Falk *et al.*, 2014), indicating that they are unlikely to only reflect the different initial numbers of recipient nymphs during the two types of food transfer tests, as well as the limited contacts between mother and nymphs during the experiment.

An interaction between clutch size and the type of food transfer shaped the proportion of recipient nymphs that became coloured during the food transfer tests (Table 1A, Fig. 2a). Specifically, the proportion of coloured nymphs was positively associated with clutch size in the sibling food transfer tests (estimate ± SE: 0.035 ± 0.015, $t_{46} = 2.43$, $P = 0.019$), but not in the maternal food provisioning tests (estimate ± SE: −0.016 ± 0.019, $t_{46} = -0.86$, $P = 0.386$). The contrasting influence of clutch size on maternal food provisioning and sibling food transfer was also present when analysing the absolute number of newly coloured recipient nymphs (Table 1B, Fig. 2b). Accordingly, the number of nymphs that had received food increased with clutch size in the case of sibling food transfer (estimate ± SE: 0.240 ± 0.028, $t_{46} = 8.635$, $P < 0.001$), but not in the case of maternal food provisioning (estimate ± SE: 0.086 ± 0.073, $t_{46} = 1.18$, $P = 0.243$). Contrary to the expectation that competition for a constant *per capita* amount of faeces increases with clutch size, the ratio of nymphs that were weakly coloured after sibling food transfer tests did not depend on clutch size ($\chi_1^2 = 0.44$, $P = 0.501$). Independent of other effects, the proportion and absolute number of nymphs that received food from family members was higher on day eight (mean ± SE; proportion = 0.55 ± 0.05; absolute = 7.75 ± 0.82) than on day four (proportion = 0.42 ± 0.05; absolute = 5.67 ± 0.69) after hatching (Table 1A and B, Fig. S2), presumably reflecting the increased nutritional needs of older nymphs (Wong & Kölliker, 2012).

The level of sibling food transfer was negatively associated with the level of maternal food provisioning after taking the influences of clutch size and the day of the respective food transfer test into account ($\rho = -0.306$, $S_{46} = 24062$, $P = 0.035$, Fig. 3). This result is in line with the hypothesis of a compensatory relationship between sibling cooperation and parental care.

Table 1 Effects of type of test (sibling food transfer or maternal food provisioning), clutch size and day of food transfer test on (A) the proportion and (B) the number of nymphs that received food from family members. Significant *P*-values are in bold print.

	(A) Proportion of nymphs coloured		(B) Number of nymphs coloured	
	Wald χ_1^2	*P*	Wald χ_1^2	*P*
Type of food transfer (TFT)	44.44	**< 0.0001**	2.72	0.0992
Clutch size (CS)	< 0.01	0.9533	19.46	**< 0.0001**
Day of food transfer test	8.19	**0.0042**	5.95	**0.0147**
TFT:CS	6.85	**0.0089**	5.33	**0.0209**

Finally, the level of food transfer among siblings was correlated with the expression of fitness-relevant traits in mothers, but not in their offspring. Specifically, higher than predicted levels of sibling food transfer were associated with increased delays in the mother's production of a 2nd clutch (Table 2A, Fig. 4a) and reduced numbers of 2nd clutch eggs (Table 2A, Fig. 4b). They were, however, not linked to the occurrence of 2nd clutch production (Table 2A), the hatching success of the 2nd clutch eggs (Table 2A) or the number of resulting nymphs (Table 2A). Higher than predicted levels of sibling food transfer were neither associated with the development time of 1st clutch offspring (Table 2B), nor with the probability of their survival until adulthood (Table 2B).

Discussion

A growing number of studies have demonstrated that the benefits of family life for offspring do not only derive from parental care, but can also arise from cooperative interactions with juvenile siblings (e.g. Botelho *et al.*, 1993; Biedermann & Taborsky, 2011; Roulin *et al.*, 2012; Yip & Rayor, 2013; Falk *et al.*, 2014). In addition to challenging the so far almost exclusive focus on parental care as the predominant mechanism promoting the emergence of family life (e.g. Clutton-Brock, 1991; Royle *et al.*, 2012), these findings prompted the question whether sibling cooperation and parental care might have jointly shaped the evolutionary transition from solitary to social life.

In this study, we showed that, in the European earwig, the level of sibling food transfer (a form of sibling cooperation characterized by a socially induced, kin-directed production of faeces that can be consumed by other group members) – but not of maternal food provisioning (a form of parental care) – increased with

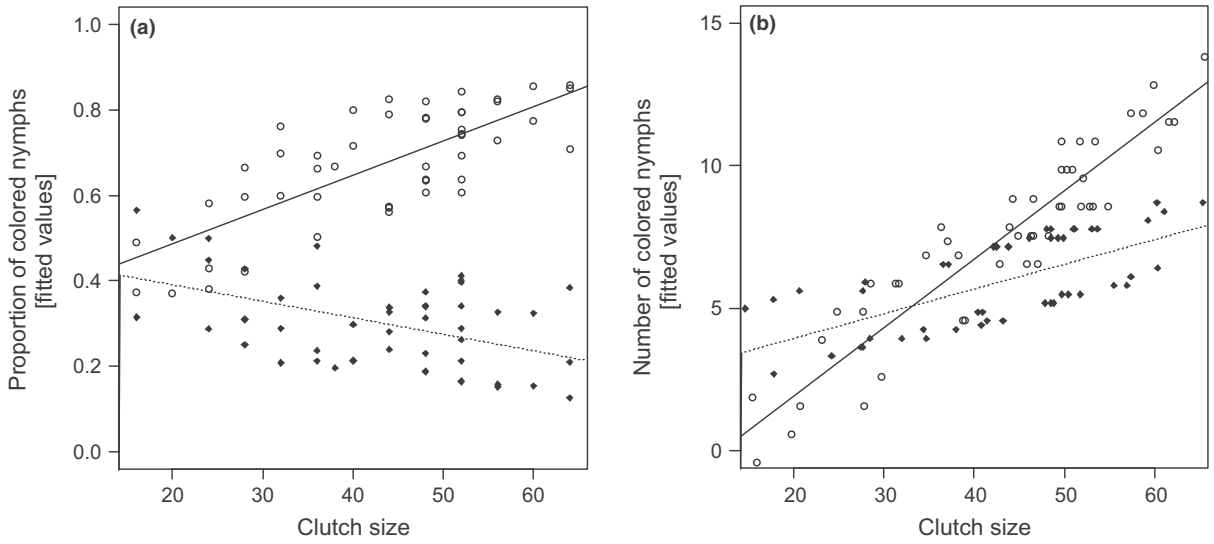

Fig. 2 Influence of clutch size on the proportion (a) and number (b) of nymphs that received food during maternal food provisioning (filled squares) and sibling food transfer (open circles).

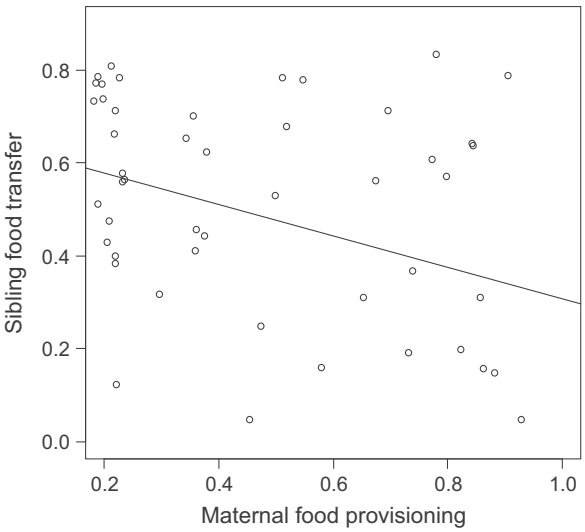

Fig. 3 Correlation of the residuals of sibling food transfer and maternal food provisioning after taking the influences of clutch size and the day of the respective food transfer test into account (see Results).

group size. Notably, this increase was not associated with a change in food distribution among offspring, a result expected under the assumption that recipient nymphs do not compete more intensively for donor faeces in larger clutches. Furthermore, higher than predicted levels of sibling food transfer (with regard to clutch size and the day of measurement) were associated with lower than predicted levels of maternal food provisioning and *vice versa*. This finding is in line with the hypothesis of a compensatory relationship between sibling food transfer and maternal food provisioning. Finally, higher than predicted levels of sibling food transfer were associated with a delayed production and reduced size of the mothers' 2nd clutch, but not with the development time and the survival of 1st clutch nymphs until adulthood, the hatching success of the 2nd clutch eggs and the number of resulting nymphs.

A compensatory association between sibling food transfer and maternal food provisioning during family life suggests that the benefits of parental care and sibling cooperation can be entangled and could have jointly promoted the early evolution of group living. Parental provisioning of offspring is a derived form of care that has been proposed to emerge from an ancestral state resembling that of contemporary precocial species (Gardner & Smiseth, 2010). In this state, benefits of food sharing and/or other forms of sibling cooperation might have played an essential role in maintaining family life, while simultaneously setting the stage for the evolution of parental provisioning, for example by providing the offspring with an additional incentive to re-aggregate after independent foraging trips which in turn could enable the parent(s) to (mass) provision their offspring more effectively. The coexistence of maternal food provisioning and sibling food transfer in earwig families suggests that sibling food transfer might currently be maintained in families of the European earwig to compensate for low levels or even the complete lack of maternal food provisioning (Mas & Kölliker, 2011; Meunier & Kölliker, 2012b; Meunier *et al.*, 2012). The overall higher prevalence of sibling food transfer as

Table 2 Effects of the deviation from the predicted level of sibling food transfer on either (A) traits of mothers and nymphs in the second clutch or (B) traits of nymphs in the first clutch. Statistical values were obtained from linear models (LM) or generalized linear models (GLM). *P*-values that remained significant after correction for multiple testing are in bold.

	Deviation from predicted sibling food transfer				
	Model	*n*	Estimate ± SE	*t*	*P*
(A) Second clutch					
Occurrence of 2nd clutch production	GLM	48	−4.06 ± 2.50	−1.63	0.111
Days between isolation and egg laying	LM	41	8.99 ± 3.63	2.48	**0.018**
Egg number	LM	48	−27.27 ± 10.57	−2.58	**0.013**
Hatching success	GLM	41	1.07 ± 1.13	0.95	0.347
Nymph number	LM	48	−8.62 ± 11.67	−0.74	0.464
(B) First clutch					
Nymph development time	LM	48	−1.16 ± 0.57	−2.05	0.047
Nymph survival until adulthood	GLM	48	0.13 ± 0.23	0.57	0.574

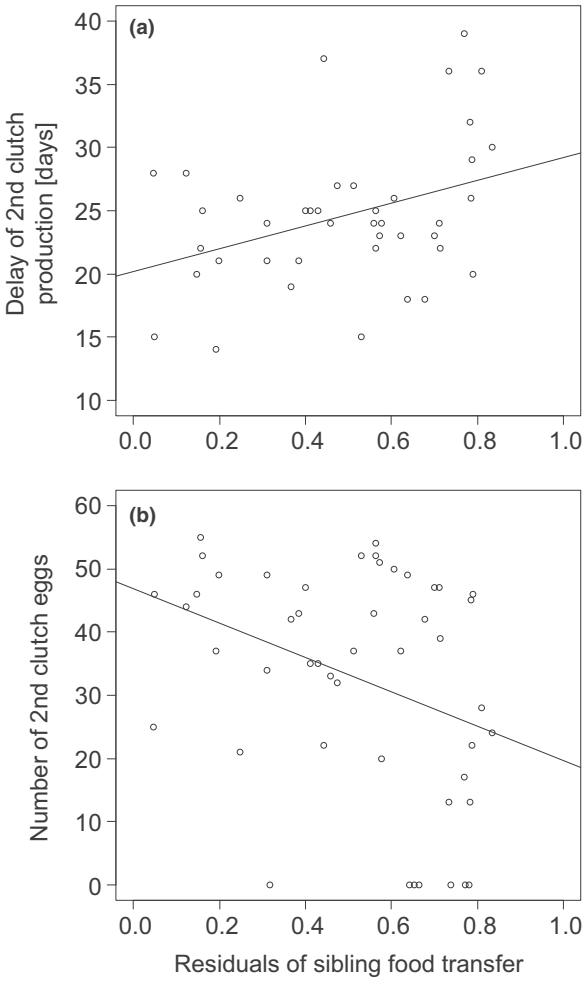

Fig. 4 Correlation of the residuals of sibling food transfer with (a) the duration from maternal isolation until 2nd clutch production and (b) the number of eggs in the 2nd clutch.

compared to maternal food provisioning is in line with this scenario, as it suggests that sibling food transfer also occurred in families in which the mother did not provision (this study), or was experimentally prevented from provisioning her offspring (Falk *et al.*, 2014). Conversely, sibling food transfer might have evolved secondarily to compensate for low levels of maternal food provisioning. This alternative scenario is, however, unlikely as the evolution of parental provisioning also drives the evolution of increased levels of sibling competition (Smiseth *et al.*, 2007; Gardner & Smiseth, 2010), which in turn should impede the evolution of sibling cooperation (Frank, 1998; West *et al.*, 2001). As a consequence, some forms of sibling cooperation, especially if they involve the exchange of resources acquired independently from parents, could be lost when the evolution of parental provisioning progresses.

Although sibling food transfer reflects a form of cooperation by donor nymphs (Falk *et al.*, 2014), both cooperative and competitive behaviours could mediate the distribution of the publicly available faeces among recipient nymphs. Cooperation is generally less likely to occur if competition between interacting individuals is high (Frank, 1998; West *et al.*, 2001). Accordingly, the incentive of offspring to share food should be inversely related to the severity of sibling competition, which in turn is classically assumed to increase with group size (Alexander, 1974; but see Shen *et al.*, 2014). Contrary to this prediction, our results showed that the level of sibling food transfer increased with group size. This increase in sibling food transfer could reflect an increased propensity of donor nymphs to transfer food to their siblings in larger clutches. Such an association could be expected if the higher number of potential donors in larger clutches ensures that the likelihood of reciprocally receiving food in times of need is increased. This in turn would lower individual costs of food sharing.

Alternatively or additionally, the association of sibling food transfer with group size could reflect increased competition of recipient nymphs in larger clutches. In this situation, the *per capita* amount of faeces cooperatively produced by donor nymphs would be independent of group size and the increase in sibling food transfer with group size would be solely based on increasing scramble competition among recipients for the publicly available faeces. Such an increase in sibling competition with clutch size could, for example, be expected if maternal investment in individual offspring decreases with increasing clutch size. In line with the hypothesis of increased competition in larger clutches,

sibling rivalry has been shown to increase with group size in earwigs (Kölliker, 2007; Meunier & Kölliker, 2012b). However, contrary to this hypothesis, we found the level of maternal food provisioning to be independent of clutch size. Likewise, the proportion of recipients that received only little food from their siblings did not increase with clutch size, indicating that competition did not lead to a more skewed distribution of food in larger clutches. Finally, the increase in sibling food transfer with clutch size could be independent of changes in nymphal behaviour with clutch size and instead simply reflect the increased absolute amount of donor faeces available to the recipients in larger clutches and/or secondary transfer of coloured food among recipient nymphs. These hypotheses are, however, unlikely to be the sole drivers of our results, as the absolute number of recipient nymphs linearly increased with clutch size (and the increased amount of faeces is thus accounted for in our sibling food transfer measurement) and because each individual cannot produce a larger amount of faeces than the amount of resources it previously ingurgitated (i.e. a nymph's faeces production cannot feed more than one sibling until satiety). The mechanism(s) underlying the increase in sibling food transfer with clutch size will be explored in further studies.

The benefits of sibling cooperation have been proposed to be an important driver of the evolution of family life (Falk *et al.*, 2014). However, we found that higher than predicted levels of sibling food transfer were neither linked to offspring survival until adulthood nor associated to their development time. One potential explanation for this apparent lack of fitness benefits for offspring is that sharing food with siblings does not augment the overall benefits of maternal care (Kölliker, 2007), but rather only allows nymphs to compensate for the detrimental effects of low levels of maternal food provisioning. In line with this hypothesis, we found that higher than predicted levels of sibling food transfer were associated with lower than predicted levels of maternal food provisioning. Moreover, Falk *et al.* (2014) observed lower levels of sibling food transfer when nymphs had the possibility to freely interact with their mother, suggesting that nymphs could prefer maternal food provisioning over sibling food transfer due to the higher quality of the maternally provided food. Alternatively, the absence of differences in survival could also reflect a limited importance of sibling food transfer (and maternal food provisioning) for nymphal survival under laboratory conditions. This could be the case as these conditions allow self-foraging in the absence of the risk of predation and consequently relax nymphal dependence on resources obtained by other family members.

Sibling cooperation by definition entails benefits for offspring, but cooperative interactions among their offspring could also benefit parents, for example by reducing offspring demand and hence allowing parents to reduce investment in parental care. However, earwig mothers tending clutches with higher than predicted levels of sibling food transfer did not produce larger 2nd clutches, despite the fact that they simultaneously showed low levels of maternal food provisioning. Interestingly, these mothers even produced fewer 2nd clutch eggs and delayed the production of their 2nd clutch longer than mothers tending nymphs that showed lower levels of sibling food transfer. Hence, mothers likely do not selectively retain resources for the production of their 2nd clutch when their 1st clutch offspring shows high levels of sibling food transfer. Instead, the combination of low levels of maternal food provisioning during 1st clutch family life and the small size and delayed production of the 2nd clutch suggests that variation in maternal investment into offspring care and future reproduction reflect differences in intrinsic female quality (Reznick *et al.*, 2000; Koch & Meunier, 2014). Whether and how such differences in female (and nymph) quality affect sibling food transfer will be investigated in further studies.

To conclude, our study reveals that maternal care and sibling cooperation are interdependent processes that together shape food acquisition by offspring in the European earwig *F. auricularia*. Our results are in line with a compensatory relationship between sibling cooperation and maternal care and thus suggest that sibling cooperation is an ancestral behaviour that can persist to mitigate the detrimental effects of low levels of parental care. More generally, our findings stress the importance of sibling cooperation among juvenile offspring in the early evolution of social life, especially if the cooperative interactions involve the transfer of resources acquired independently of parents.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Effects of the deviation from the predicted level of maternal food provisioning (MFP) on (A) the development time and the survival of 1st-clutch nymphs until adulthood.

Figure S1 Proportion (a) and number (b) of colored recipient nymphs after the maternal food provisioning (MFP) and sibling food transfer (SFT) tests.

Figure S2 Proportion (a) and absolute number (b) of recipient nymphs colored after the food transfer tests on day four (D4) and day eight (D8), respectively.

Data deposited at Dryad: doi:10.5061/dryad.5rg54.

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Original Article

Maternal condition determines offspring behavior toward family members in the European earwig

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Parental care confers benefits to juveniles but is usually associated with substantial costs for parents. These costs often depend on parental condition, which is thus considered as a key determinant of the level of parental care expressed during family life. However, how parental condition affects the behaviors that juveniles express toward their siblings and parents remains poorly explored. Here, we investigated these questions in the European earwig *Forficula auricularia*, an insect in which mothers provide extensive forms of care to their juveniles. We measured maternal body condition at egg hatching, subsequently manipulated maternal nutritional state, and finally assessed both food transfer among siblings and the nature of mother–offspring interactions. We also considered variation in brood size, an important parameter in family interactions. We found that food transfer among siblings increased with brood size when the tending mothers were in a deteriorated nutritional state. This effect was masked when the nutritional state of mothers was enhanced. The frequency of care-related behaviors that juveniles expressed toward their mother was higher when she was in a deteriorated rather than an enhanced nutritional state, while it overall increased with brood size. Finally, increasing values of maternal body condition entailed a shift from a positive to a negative association between maternal care behaviors and brood size, but only when the mothers' nutritional state was deteriorated. Overall, our results demonstrate that parental condition and brood size do not only affect parental behaviors but can also be important and entangled drivers of offspring behaviors during family life.

Key words: ***Forficula auricularia*, parental care, precocial species, sibling rivalry, social evolution.**

INTRODUCTION

Parental care is a common and taxonomically widespread phenomenon in nature and usually confers substantial benefits to the tended juveniles (Royle et al. 2012). However, providing care often entails high costs for parents, such as an increased loss of energy or an elevated risks of infection and predation (Alonso-Alvarez and Velando 2012), which can reduce their ability to invest in future reproduction (Trivers 1972). Because parents in a bad condition are expected to incur higher costs of care than parents in a good condition (Hinde et al. 2010), they are generally predicted to adjust the expression of parental care to their body condition as well as to short-term changes in their nutritional state in order to maximize their reproductive success (Bateson 1994). Several studies provided support for this prediction (e.g., Markman et al. 2002; Gorman and Nager 2003; Laurien-Kehnen and Trillmich 2004; Bleeker et al. 2005; Segers et al. 2011; Wong and Kölliker 2012), thus revealing the central importance of parental body condition and nutritional state in the expression of parental care. For instance, mothers in a poor nutritional state were shown to reduce parental care toward their current offspring in the mouthbrooding cichlid, *Simochromis pleurospilus* (Segers et al. 2011), whereas food-deprived guinea pig (*Cavia porcellus*) mothers prolonged the expression of nursing behavior while maintaining a constant body condition, thus ultimately reducing the growth rate of their pups (Laurien-Kehnen and Trillmich 2004). Conversely, artificial food supplementation with a sucrose solution allowed parent Palestine sunbirds (*Nectarinia osea*) to increase the rate at which they provisioned their nestlings with arthropods (Markman et al. 2002).

The optimal level of care from the point of view of the parents, however, does not necessarily coincide with the optimal level of care from the offspring's point of view. Because of relatedness asymmetries among family members, offspring often behave more selfishly than their parents desire (Trivers 1974; see also Mock and Forbes 1992), both by trying to monopolize resources at the expense of their siblings (sibling rivalry; Mock and Parker 1997; Roulin and Dreiss 2012), as well as by manipulating their parents

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into increasing their parental investment (Kilner and Hinde 2012). The resulting parent–offspring conflict over the amount of parental investment into care compels parents and offspring to monitor each other's state (Royle et al. 2002; Morales and Velando 2013) and to adjust their strategy of providing and demanding care accordingly (e.g., Godfray and Johnstone 2000; Parker et al. 2002; Smiseth et al. 2003). By analogy, parental body condition and nutritional state should not only influence the behavior of tending parents but might also affect how offspring interact with each other, as well as how they act toward their parents (Bateson 1994).

Although a number of studies have tested the influence of parental body condition on the expression of parental care and the (begging) behavior of offspring toward their parents (see above), it remains poorly explored how parental condition and its changes during family life affect the behavior of juveniles toward their own siblings (but see White et al. 2010; Wong, Lucas, et al. 2014 for effects on aggressive behavior). Here, we investigated this effect in the European earwig *Forficula auricularia*. In this insect species, mothers provide nonobligatory forms of care to their mobile offspring (called nymphs) for several weeks after hatching (Lamb 1976a). Maternal care comprises multiple behaviors including the provisioning of nymphs (Lamb 1976b; Staerkle and Kölliker 2008), the amount of which is reduced when mothers had limited access to food resources during family life (Wong and Kölliker 2012). While such condition-dependent behavioral changes in maternal care might enable nymphs to indirectly monitor short-term changes in their mother's condition, nymphs can also assess maternal condition based on cues/signals that are encoded in the profile of the mother's cuticular hydrocarbons (Wong, Lucas, et al. 2014). Interestingly, maternal presence and posthatching care are not obligatory for offspring survival (Lamb 1976b; Kölliker 2007; Kölliker and Vancassel 2007). Earwig nymphs do not exclusively rely on maternal provisioning, but may also forage independently soon after hatching (Lamb 1976b; Wong and Kölliker 2012) and obtain food from their siblings (Falk et al. 2014; Kramer et al. 2015). This food transfer among juveniles is predominantly mediated by active allocoprophagy, a process defined as a socially induced increase in feces production by donor nymphs and the subsequent consumption of these feces by recipient siblings (Falk et al. 2014).

To unravel whether offspring behaviors toward family members were associated with maternal condition at egg hatching (initial body condition, a proxy for the mother's long-term energetic state) and/or the posthatching access of mothers to food resources (nutritional state, a proxy for short-term changes in the satiety level), we first determined the body condition of mothers at egg hatching, subsequently manipulated their nutritional state during 4 days and then assessed sibling food transfer (SFT) and mother–offspring interactions. If the mother's nutritional state influenced the behavior of offspring toward their siblings and toward their mother, we would expect 1) a decrease in food transfer among the nymphs, as well as 2) a lower frequency of care-related offspring behaviors (such as begging) in families tended by mothers in an enhanced as compared with a deteriorated nutritional state. This is because mothers in an enhanced nutritional state typically show higher levels of maternal care (Wong and Kölliker 2012). We also expected that mothers with a high body condition at egg hatching could generally afford higher levels of care than mothers with a low initial body condition. Accordingly, we predicted that 3) a high maternal body condition at egg hatching would reinforce the positive effects of an enhanced nutritional state on the level of parental care and 4) allow, in comparison with a low initial body condition, higher levels of care when a mother's current state is deteriorated. Note that we also considered (differences in) family size in our analyses of the effects of maternal condition and nutritional state on family interactions because group size is generally assumed to affect the competition among group members (Alexander 1974; Shen et al. 2014) and could thus modify reactions of family members to changes in maternal condition or state. Moreover, family size has been suggested to influence mortality and developmental rates of nymphs in European earwigs (Kölliker 2007; Meunier et al. 2012; Meunier and Kölliker 2012) and is linked to the level of food transfer among their offspring (Kramer et al. 2015).

MATERIALS AND METHODS

Study animals and laboratory rearing

The adult *F. auricularia* females used in the experiment descended from 80 female and 73 male earwigs collected in September 2012 in a natural population in Dolcedo, Italy. These individuals were maintained in the laboratory under standard rearing conditions (detailed in Koch and Meunier 2014) for 2 generations. The experiment comprised of 2 randomly chosen, independent subsets of adult females and their respective first brood of offspring derived from the first (n = 19 families used in 2013) and the second (n = 29 families used in 2014) filial generation, respectively. These subsets neither differed with respect to maternal body size, weight, or body condition at egg hatching (details on these measurements below) nor with regard to brood size (see Manova results).

Experimental setup

A total of 48 families were used to disentangle the effects of maternal body condition at egg hatching and subsequent changes in maternal nutritional state on the level of SFT and the frequency of mother–offspring interactions. The corresponding experimental design is detailed in Figure 1. Females had no access to food between egg laying and hatching (mean duration ± standard error [SE]: 33 ± 2 days), as *F. auricularia* females generally do not consume food during that period of time (Kölliker 2007). One day after egg hatching, families were randomly attributed to one of 2 treatments. In the first treatment (n = 24 families; 9 in 2013 and 15 in 2014), mothers did not receive any food before the behavioral observations (i.e., until day 4, deteriorated state). In the second treatment, mothers had access to uncolored food (naturally yellow-colored flower pollen formed into pellets; Hochland Bio-Blütenpollen, Hoyer GmbH, Polling, Germany) for 20 min on the first and the fourth day after hatching (enhanced state; n = 23 families; 9 in 2013 and 14 in 2014). As intended, the nutritional state of females in the first treatment deteriorated, whereas the state of females in second treatment improved (see Supplementary Material for details). In both treatments, nymphs were provided with uncolored food for 1.5 h on the first and second day after hatching. No food was provided on the third day after hatching to increase their foraging and solicitation behavior toward mothers and siblings on the following day (Staerkle and Kölliker 2008; Falk et al. 2014). On day 4 after hatching, the nature and frequency of mother–offspring interactions and the level of SFT were investigated in each family (see details below).

Throughout the experiment, families were kept in their original, medium-sized Petri dish ($9 \times 2\,cm^2$) with humid sand as ground material and a plastic tube as shelter. During offspring feeding and (where applicable) to provide them with food, mothers were

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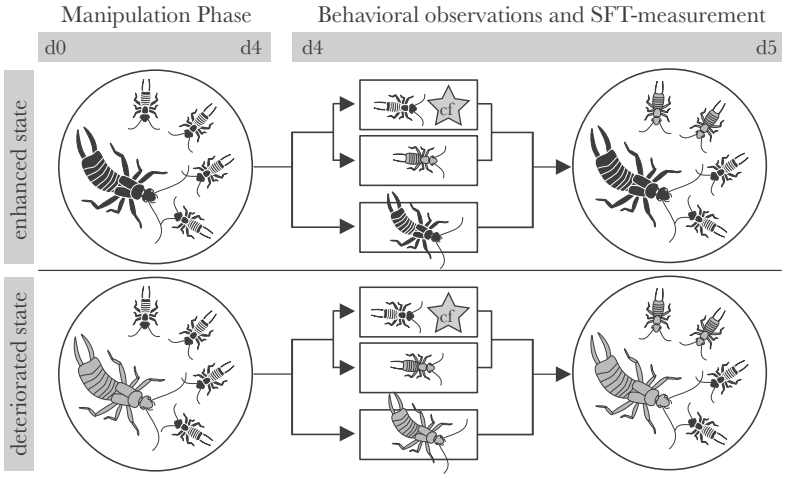

Figure 1
Experimental setup. Female nutritional state was manipulated by selectively feeding (enhanced) or starving (deteriorated) her prior to the behavioral tests. SFT was measured by providing half of the nymphs (called donor nymphs) with colored food (indicated with "cf"), then reassembling these newly colored nymphs with their remaining, food-deprived siblings (called recipient nymphs) and their mother, allowing family interactions overnight and finally counting the number of recipient nymphs that ingested the colored food provided by the donor nymphs. Gray individuals were food deprived in the corresponding experimental phase.

isolated in a small Petri dish ($5.5 \times 1.2\,cm^2$) that only contained humid sand as ground material. Brood size was established by counting the number of nymphs 1 day after the first egg hatching had been observed. To control that our treatments induced changes in maternal nutritional state, females were weighed on the first and fourth day after hatching (before the onset of behavioral observations) to the nearest 0.01 mg using a microscale (model MYA5; PESCALE, Bisingen, Germany). After the behavioral observations, females were sedated with CO_2 and their eye distance was measured to the nearest 0.001 cm using a camera coupled to a binocular (Leica DFC425, Leica Microsystems Ltd, Heerbrugg, Switzerland) and the software *Leica Application Suite 4.5.0*. Using eye distance as a measure of body length together with female weight at egg hatching, we calculated the initial body condition for each female based on the "scaled mass index" (Peig and Green 2009; Peig and Green 2010). In brief, this index standardizes body mass at a fixed value of a linear body measurement based on the scaling relationship between these measures (Peig and Green 2009). Accordingly, this index indicates which mass a particular female would have at the average eye distance. Note that initial body condition, brood size, as well as maternal weight and size at egg hatching did not differ between the "enhanced state" and "deteriorated state" treatments in both experimental seasons (Manova; interaction treatment:season: $\Lambda_{Pillai,1} = 0.052$, $P = 0.700$; treatment: $\Lambda_{Pillai,1} = 0.032$, $P = 0.848$; season: $\Lambda_{Pillai,1} = 0.106$, $P = 0.308$).

Assessments of SFT and mother–offspring interactions

The measurement of SFT relied on the fact that ingested colored food remains visible through the partially transparent cuticle of young *F. auricularia* nymphs (Staerkle and Kölliker 2008; Falk et al. 2014). SFT was measured in 3 successive steps according to a previously established, standard protocol (Kramer et al. 2015). First, we provided one-half of the nymphs (called donor nymphs) with colored food (naturally yellow-colored pollen mixed with a blue food dye; Dekoback, Online Ideen GmbH, Germany) for 1 h in a small Petri dish, while the remaining nymphs were starved separately. Simultaneously, mothers were either starved or had access to uncolored pollen for 20 min (see treatments aforementioned). Note that temporary separations of nymphs from their mother commonly occur in nature during independent foraging trips (Lamb 1976b). In the second step, we reassembled the newly colored donor nymphs with their remaining siblings (called recipient nymphs) and their mother to allow family interactions. Fifteen hours later, we finally counted the number of recipient nymphs that ingested the colored food provided by the donor nymphs using a stereomicroscope. To be able to discriminate between donors and recipients nymphs, either all donor (n = 27 trials) or all recipient nymphs (n = 20 trials) of a given family were chosen at random and marked by clipping off the distal third of their right cercus (Wong and Kölliker 2013) prior to the experiment. Nymphal coloration was scored by 1 single observer and blindly with regard to the treatment of the mother throughout the experiment. The level of SFT (measured as the proportion of recipient nymphs that received colored food from siblings) was independent of marking (Wilcoxon rank sum test; $W = 263$, $P = 0.889$).

The behavioral interactions between mothers and their offspring were assessed in the course of the measurement of SFT and categorized into care-related behaviors expressed by offspring, but also care and aggressive behaviors expressed by mothers. The behaviors were recorded using a scan sampling approach (1 observation every 5 min for 45 min, i.e., 10 scans in total per replicate) starting 15 min after the family members had been reassembled in their original Petri dish to allow food transfer (see above). Care-related offspring behaviors comprised 1) "licking" behaviors, during which nymphs manipulate the intersegmental skin between abdominal segments and/or the leg-joints of the mother with their mouthparts, 2) begging behaviors, during which nymphs try to establish mouth-to-mouth-contact with the female, and 3) mouth-to-mouth contacts. The number of occurrences of each of these behaviors (i.e., the number of times a given behavior was performed by at least 1 nymph) was then summed up across all 10 scans obtain the overall frequency of care-related offspring behaviors. Maternal care behavior was defined following Mas and Kölliker (2011) and

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was recorded as the sum of 1) antennal contacts with the nymphs, 2) allogrooming during which the mother manipulated nymphs with her mouth parts, and 3) mouth-to-mouth contacts. Mouth-to-mouth contact was considered as both female and nymph behavior because it required coaction of the interacting individuals. Finally, aggressive behaviors expressed by mothers were the sum of threat displays, during which the female raised her forceps in the direction of a nymph, and abdomen shaking, a behavior allowing females to cast off riding nymphs. As this study only focuses on mother–offspring interactions, we did not analyze self-directed female behaviors such as resting, self-grooming, and exploring. All behavioral observations were conducted blindly with respect to the nutritional state of the female. Note that all of the above detailed measurements were based on half of the nymphs per family (half of the nymphs were haphazardly chosen and removed from their families before the onset of the observations reported in this study, and these nymphs were used in a different experiment; data not shown).

Statistical analyses

The effects of initial body condition, offspring number, and nutritional state on SFT and mother–offspring interactions were tested in 3 generalized linear models (GLMs). In these models, initial body condition (continuous), brood size (continuous), nutritional state (enhanced or deteriorated; bimodal), and all their interactions were entered as explanatory variables. As the experiment was conducted in 2 consecutive seasons, we additionally entered "season" as a bimodal (2013 and 2014) explanatory variable in all models to account for potential differences caused by this confounding factor. We initially also included the interaction between "season" and "nutritional state" into our models, but subsequently removed it because it was never significant (all $P < 0.128$). The frequency of maternal care behaviors and the relative frequency of care-related nymphal behaviors were analyzed in 2 separate GLMs with a Poisson error distribution corrected for overdispersion. We used the relative instead of the absolute frequency of care-related nymphal behaviors (i.e., the absolute frequency divided by the brood size) to avoid a potentially confounding effect of differences in nymph number on this measure of offspring behavior. The proportion of recipient nymphs colored after the SFT test (entered as odds ratio using the "cbind"-function in R) was analyzed in a GLM with binomial error distribution corrected for overdispersion. Note that we did not statistically analyze the frequency of female aggressions against her offspring, as such aggressions occurred infrequently, both within broods (none of the 47 families featured more than 1 aggression) and across broods (aggressive behavior was only observed in 5 of 47 families).

All statistical analyses were performed using the statistics software R 3.0.3 (http://www.r-project.org/). Mixed model analyses were implemented using the package *lme4*. Significance levels of effects in these models were assessed using the packages *car* (*Anova* function) and *lmerTest* (*summary* function). Note that we centered "brood size" and "initial body condition" on their mean to avoid any model bias due to collinearity between these explanatory variables (variance inflation factor < 5.5 after centering in all models). All statistical models were simplified stepwise by removing nonsignificant interaction terms (all $P > 0.187$), as retaining these terms can bias estimates of other effects in the model (Engqvist 2005). A log-likelihood ratio (LR) test was used to test the explanatory power of each model after the removal of a variable. Where applicable, models were checked for normality of residuals and homogeneity of variance before and after the model selection procedure. Finally, interactions between continuous variables were plotted using the package *effects* to display the predicted relationship between the response variable and 1 explanatory variable for different, fixed values of the interacting variable(s) (details in Fox 2003).

RESULTS

Maternal nutritional state influenced the exchange of food among her offspring, but only through an interaction with brood size (Figure 2; Table 1a). Specifically, SFT increased with brood size when the mother's nutritional state was deteriorated (model estimate ± SE: 0.048 ± 0.016, $t_{46} = 3.073$, $P = 0.004$), whereas this association disappeared when maternal nutritional state was enhanced (estimate ± SE: -0.0003 ± 0.0163, $t_{46} = -0.021$, $P = 0.983$). Overall, the level of food transfer did not differ among clutches that had been tended by females in a deteriorated or enhanced state, respectively (Table 1a). Notably, SFT was independent of the initial body condition of the mother (Table 1a).

The relative frequency of care-related behaviors expressed by nymphs was overall higher when mothers had a deteriorated compared with an enhanced nutritional state (Figure 3a; Table 1b), but was independent of brood size, the initial body condition of the mother, and any interactions between the 3 tested variables (Table 1b). In contrast, a triple interaction between the initial body condition of the mother, brood size, and her nutritional state influenced the frequency of care-related behaviors expressed by mothers (Table 1c). When the nutritional state of mothers was deteriorated, the frequency of care behaviors increased with brood size if they initially had been in bad condition, but surprisingly decreased with brood size if they had been in good condition (Figure 4; interaction between initial body condition and brood size: LR $\chi^2_1 = 5.35$, $P = 0.021$; initial body condition: LR $\chi^2_1 = 0.97$, $P = 0.325$; brood size: LR $\chi^2_1 = 0.30$, $P = 0.583$). In contrast, when the nutritional state of mothers was enhanced, their initial body condition did not influence the frequency of maternal care behaviors, irrespective of brood size (interaction between initial body condition and brood size: LR $\chi^2_1 = 1.74$, $P = 0.187$; initial body condition: LR $\chi^2_1 = 1.08$, $P = 0.298$; brood size: LR $\chi^2_1 = 3.26$, $P = 0.071$). Finally, aggressive behavior of mothers against their offspring overall occurred infrequently and were distributed across seasons and experimental treatments without any obvious pattern (occurrences per family; "enhanced state": 2014 = 0, 2013 = 2; "deteriorated state": 2014 = 2, 2013 = 1). Note that season (2014 or 2013) never had a significant effect in any of our models (Table 1).

DISCUSSION

In this study, we showed that behavioral interactions among family members in the European earwig *F. auricularia* reflect an interplay of the mother's current nutritional state, her condition at offspring emergence, and the number of her offspring. Our data revealed that SFT increased with brood size when the female was in a bad nutritional state, but was independent of brood size when she was in an enhanced nutritional state. The level of SFT was, however, not linked to the female's condition at egg hatching. We also found that the expression of care-related behaviors by offspring was overall higher if the mother was in a deteriorated rather than an enhanced nutritional state. Finally, regarding maternal care, our results demonstrated that the frequency of caring behaviors—but likely not the rare occurrence of aggressions—was shaped by an interaction between the current state of the mother, her body condition at egg

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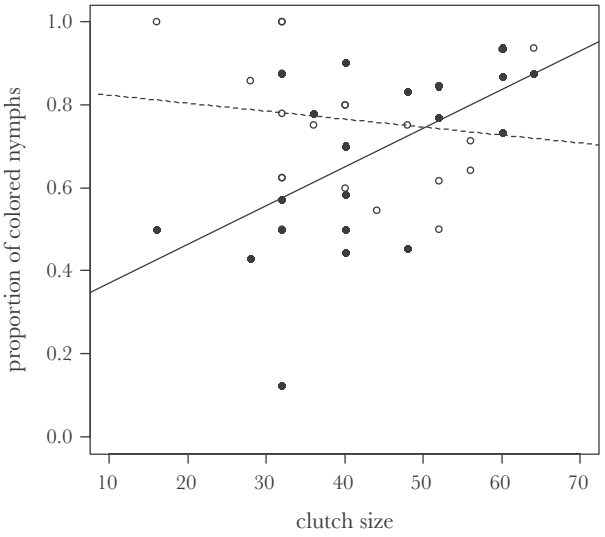

Figure 2
Influence of brood size on the proportion of recipient nymphs that received food during SFT tests conducted when maternal nutritional state was enhanced (open circles, dashed line) or deteriorated (filled circles, solid line). Note that the discrepancy between the number of visible points and the sample size is due to the overlap of some data points.

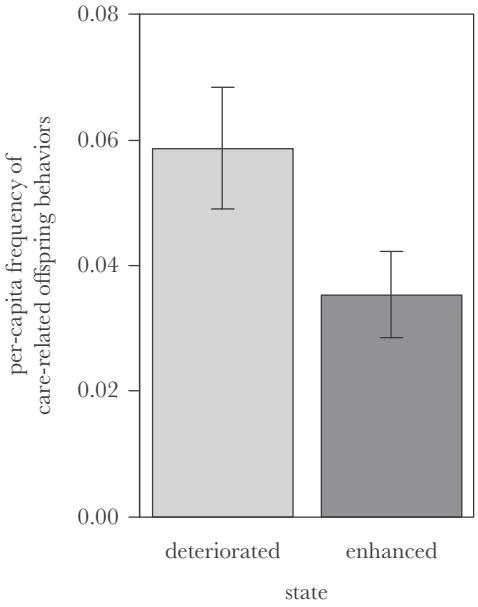

Figure 3
Effects of the mother's nutritional state on the frequency of care-related behaviors expressed by nymphs.

Table 1
Effects of the initial body condition of mothers, their brood size, and their nutritional state (enhanced or deteriorated) as well as the experimental season on (a) the level of SFT (measured as the proportion of recipient nymphs that received colored food from their siblings), (b) the frequency of care-related nymph behaviors, and (c) the frequency of maternal care behaviors

	(a) SFT		(b) Nymph behavior		(c) Maternal care	
	χ^2_1	*P*	χ^2_1	*P*	χ^2_1	*P*
IBC	0.57	0.449	0.63	0.429	0.21	0.649
BS	4.49	**0.034**	0.10	0.755	1.35	0.245
NS	0.89	0.345	3.95	**0.047**	>0.01	0.937
Season	0.11	0.736	1.92	0.166	0.13	0.716
IBC:BS	0.68	0.409	>0.01	0.933	>0.01	0.982
IBC:NS	0.14	0.708	0.02	0.900	1.68	0.196
BS:NS	4.82	**0.028**	0.14	0.707	2.99	0.084
IBC:BS:NS	0.20	0.653	0.24	0.624	6.24	**0.013**
Type of model/ error family	GLM/binomial		GLM/Poisson		GLM/Poisson	

Note that we report the results of the full models here to facilitate comparisons among them. The (qualitatively unchanged) results of the corresponding reduced models can be found in Supplementary Table S1. Significant *P* values are highlighted in bold print. BS, brood size; IBC, initial body condition; NS, nutritional state.

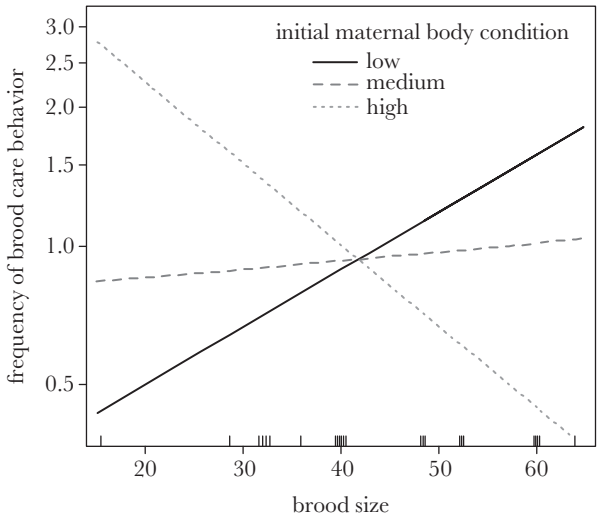

Figure 4
Interacting effect of the initial maternal body condition and brood size on the frequency of care behaviors of mothers in a deteriorated nutritional state. To illustrate the interaction, regression lines are given for an average value of the initial maternal condition (median = 0.0464; dashed line) as well as for a comparatively low (1st quartile = 0.0442; solid line) and high (3rd quartile = 0.0501; dotted line) value, respectively.

hatching, and brood size. If the mother was in a deteriorated state at the time of measurement, the frequency of maternal care behaviors increased with brood size if she had also been in a bad condition at egg hatching, but surprisingly decreased with brood size when she had been in a good initial condition. Notably, this interactive effect was masked when the female was in an enhanced nutritional state.

Our results are in line with the prediction that a mother's nutritional state shapes the behavioral interactions among her offspring. When mothers were in a deteriorated state (and thus unable to provision their offspring), the increase of SFT with brood size could reflect a density-dependent increase of competition among recipients for publicly available feces and/or brood size–dependent changes in the propensity of donors to produce these feces (Kramer et al. 2015). Conversely, the absence of such an association when mothers were in an enhanced state and thus able to provision their offspring might reflect that females provided sufficient levels of care to limit selfishness among offspring that react to cues of female condition (Wong, Lucas, et al. 2014; Wong, Meunier, et al. 2014)

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and/or to mask the above described density-dependent effects. Interestingly, such a masking effect has recently been described in the burying beetle *Nicrophorus vespilloides*, where a density-dependent shift from cooperation to competition was only evident in the absence of parental care (Schrader et al. 2015). Further experiments are needed to differentiate among the above possibilities in earwigs. Nevertheless, the deteriorated state of mothers is unlikely to have specifically triggered a density-dependent increase of feces production by donor nymphs, as the overall level of SFT was found to be independent of female state. Instead, a deteriorated maternal state is likely to increase the competition among recipient nymphs, which might in turn reduce (rather than increase) the feces production of donor nymphs. The fact that the differences in the level of food transfer between treatments were most pronounced in small broods suggests that the reduction in feces production might be larger in these broods. Hence, although SFT can possibly mitigate detrimental effects of low maternal care by providing access to additional resources in the form of feces (Kramer et al. 2015), its extent might ultimately be limited because donor nymphs neither seem to adjust the production of feces directly to their siblings' nutritional need (Falk et al. 2014) nor indirectly by monitoring and reacting to their mother's condition (this study).

The frequency of care-related behaviors that offspring directed toward their mother was higher when she was in a deteriorated rather than an enhanced nutritional state. This result is in line with the assumption of increased offspring begging in broods tended by mothers in poor condition. In this situation, offspring likely increased their begging efforts either cooperatively (e.g., Bell 2007; Madden et al. 2009) or competitively (e.g., Neuenschwander et al. 2003; Smiseth et al. 2003) to elicit sufficient levels of maternal care. Irrespective of the mediating mechanisms, the effects of maternal state on care-related offspring behaviors, as well as on the food transfer among these offspring, illustrate that offspring behaviors during family life are flexible and change according to their mother's condition.

Maternal state did not only shape sibling interactions but also affected the frequency of care-behaviors mothers expressed toward their offspring. When the nutritional state of mothers was deteriorated (but not when it was enhanced), our data showed that increasing values of maternal body condition at egg hatching entailed a shift from a positive to a negative association between maternal care and brood size. The increase of maternal care with brood size in families tended by females with both low initial body condition and deteriorated nutritional state suggests a trade-off between providing care and somatic maintenance that depends on the number—and thus on the value—of current offspring. Accordingly, females tending small broods might favor somatic maintenance over providing (some forms of) care, for example, to retain the ability to provide other forms of care such as predator defense (Bateson 1994) or a prospect of future reproduction (Thorogood et al. 2011), whereas females tending large broods might do the opposite (and possibly forfeit chances of future reproduction). In line with this latter reasoning, stitchbird or hihi (*Notiomystis cincta*) parents were shown to be largely insensitive to the experimentally enhanced begging displays of their brood when they attempted 2 breeding attempts in 1 reproductive season, but responded with an increased rate of nestling provisioning when they bred just once (Thorogood et al. 2011).

Surprisingly, the trade-off between providing posthatching care, somatic maintenance, and the prospects of future reproduction seemed to be differently solved by mothers in a deteriorated nutritional state that had featured a high body condition at offspring emergence. Under these conditions, maternal care decreased with brood size. This result could reflect that mothers with a high initial body condition generally favor future over (additional) current reproductive investment (McNamara et al. 2009), but are nevertheless capable of providing high levels of care to small broods without compromising their prospects of future reproduction. Future studies should investigate the nature and adaptive significance of this apparent trade-off between self-maintenance, care, and future reproduction. However, our findings reveal that, independent of the underlying mechanisms, females are not forced into this trade-off if their nutritional state is enhanced after offspring emergence.

In summary, we demonstrated that maternal condition did not only influence parental care but also affected interactions among juveniles and the behaviors they expressed toward their mother. Moreover, we showed that the influence of the mother's current nutritional state on offspring behaviors critically depended on both, her body condition at offspring emergence and the number of offspring she tended. These results thus call for a better integration of female nutritional state and quality in studies on behavioral interactions between parents and offspring as well as among offspring. More generally, our findings illustrate that life-history traits and environmental conditions interact to shape the complex interplay of behaviors characteristic for family life.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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Original Article

Growing up with feces: benefits of allo-coprophagy in families of the European earwig

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An important issue in the evolution of group living is the risk of pathogen and predator exposure entailed by the inherent accumulation of feces within a nesting site. While many group living species limit this risk by cleaning the nest, others do not, raising questions about the benefits of maintaining feces in the nest and their importance in social evolution. Here, we investigated whether one of these benefits could be mediated by coprophagy in families of the European earwig, *Forficula auricularia*. In this insect species, mothers and mobile juveniles (nymphs) line their nests with feces and consume them. In a first experiment, we tested whether access to feces produced by either nymphs or mothers affects nymph survival in both presence and absence of food. The results showed that access to sibling feces, but not mother feces, enhanced offspring survival under food deprivation. Such an effect did not occur when regular food was available. We then conducted a food-choice experiment to reveal whether nymphs prefer food to feces, and if they discriminate between feces from their mother, unrelated adult females, unrelated nymphs, or their siblings. We found that offspring generally preferred regular food to feces, but nevertheless always consumed some feces. By contrast, nymphs showed no preference between related sibling or mother feces and did not discriminate between feces from related and unrelated individuals. Overall, our results suggest that the benefits of coprophagy could favor the maintenance of feces within the nest and promote the evolution of social life.

Key words: **frass, insect, precocial, sibling cooperation, social evolution.**

INTRODUCTION

Although defecation is an essential process to dispose nutritional waste, the accumulation of its product in a nesting site may entail costs for the surrounding individuals that are often thought to hamper the evolution of group living (Weiss 2006). This is because a wide range of pathogenic bacteria and fungi are known to use feces as a substrate for their development (Bailey 1955; Bucher 1957), and because feces releases kairomones that can be used by predators to locate and attack their prey (Vet and Dicke 1992; Agelopoulos et al. 1995; Steidle and Fischer 2000). These feces-related risks of infection and predator attacks remain limited in species with low nest fidelity (Weiss 2006; Quan et al. 2015). However, they can grow dramatically when organismic density is high and/or living space is confined, such as in nest-dwelling species (Schmid-Hempel 1998; Weiss 2006; Jackson and Hart 2009). This is why the emergence and maintenance of group living has long been thought to require the expression of sanitation behaviors (Meunier 2015), such as expelling feces from the nest (Thomson 1934; Michener 1974; Weiss 2003; Biedermann and Taborsky 2011) or limiting defecation to a single location (Dethier 1980; Zuri and Terkel 1998; Georgiev 2009; Farji-Brener et al. 2016).

A growing number of studies have recently shown, however, that keeping feces within a nesting site may provide benefits for group members and could thus promote the evolution of social life. One of these benefits relies on the antimicrobial activities possibly exhibited by feces material. This activity has been demonstrated in multiple species that coat their nest/colony with feces to prevent the growth of pathogens, such as in the dampwood termite *Zootermopsis angusticollis*, the wood cockroach *Cryptocercus punctulatus*, the burying beetle *Nicrophorus vespilloides*, and the European earwig *Forficula auricularia* (Rosengaus et al. 1998, 2013; Reavey et al. 2014; Diehl et al. 2015). A second benefit of keeping feces in a nesting site is that it may foster the consumption of feces produced by conspecifics—a

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behavior called allo-coprophagy—and thus facilitate the exchange of symbionts between group members. This phenomenon has been well studied in wood-feeding termites, in which allo-coprophagy and (mostly) proctodeal trophallaxis were shown to mediate the transfer of mutualistic gut bacteria that are essential for digesting specific foods (Cleveland 1925; Nalepa et al. 2001; Engel and Moran 2013; Mirabito and Rosengaus 2016). Finally, facilitated access to allo-coprophagy may also provide nutritional benefits for group members. Such benefits have been reported in the sub-social German cockroach *Blattella germanica*, in which the consumption of fecal pellets produced by conspecifics increased individuals' resistance against starvation (Kopanic et al. 2001). Coprophagy has also been reported in many solitary species, where it allows for the re-ingestion of otherwise poor food sources (Hirakawa 2002) or simply supplements the regular diet (Nilsson 1983).

Interestingly, the benefits of allo-coprophagy could also play a major role in the evolution of family life by mediating the often overlooked benefits of sibling interactions. For decades, the nature of sibling interactions was typically ranged from mere neutral tolerance to costly rivalry over parental resources (Mock and Parker 1997; Roulin and Dreiss 2012). Sibling interactions were thus generally considered as a potential inhibitor rather than a promoter of the emergence and maintenance of family life (Trivers 1972; Royle et al. 2014). However, a growing number of studies have recently indicated that sibling interactions could provide benefits to juveniles through food exchange during family life (reviewed in Roulin and Dreiss 2012). The consumption of feces during family life could be a key mediator of this food exchange, such as in the European earwig *F. auricularia*. In this species with facultative maternal care (Kölliker 2007; Meunier and Kölliker 2012; Thesing et al. 2015), juveniles (called nymphs) have been shown to increase feces production in the proximity of their siblings, thereby promoting the sharing of food resources through allo-coprophagy (Falk et al. 2014; Kramer et al. 2015; Kramer and Meunier 2016). Nevertheless, the nutritional and/or non-nutritional benefits of allo-coprophagy for nymphs and thus their possible role in the emergence and maintenance of family life, as well as the mechanisms regulating the expression of allo-coprophagy, remain unknown in this species.

In this study, we investigated the benefits of and the mechanisms regulating allo-coprophagy in nymphs of the European earwig *F. auricularia*. In a first experiment, we tested whether allo-coprophagy enhances nymphs' survival and whether this effect depends on their access to a regular food source. To this end, we manipulated nymph access to nymphal/maternal feces and to a regular food source and then measured nymph survival rates over 25 days. If access to feces provided nutritional benefits, we expected to find higher survival rates in nymphs that were provided with feces than in nymphs that were not, and that this effect would be stronger in the absence of a regular food source. Conversely, if access to feces provided non-nutritional benefits, we expected nymphs to survive better in the presence of mother and/or nymph feces, independent of the presence of a regular food source. In the second experiment, we tested whether the expression of allo-coprophagy and thus its potential nutritional and non-nutritional benefits in nymphs depend on whether the feces are produced by other nymphs or by adult mothers and on the feces producer's relatedness (and/or familiarity). Specifically, we set up a series of paired food-choice tests in which nymphs could choose between regular food and feces produced by an adult female or a nymph, which were either unrelated or related to the focal nymph. If feces were only used as a food source under harsh circumstances, we expected nymphs to prefer the consumption of regular food to any type of feces, as well as to prefer nymphal (i.e., possibly less digested, and hence more nutritious) over maternal feces. Conversely, if coprophagy was also used as a mediator of symbiotic exchanges (i.e., a type of non-nutritional benefit) and these exchanges are family specific, we expected the nymphs to prefer the consumption of feces from related donors over feces produced by unrelated individuals.

MATERIALS AND METHODS

Animal origin and maintenance

Our 2 experiments involved a total of 58 clutches produced by a second laboratory-born generation of females field sampled in 2012 in Dolcedo, Italy (experiment 1: $n = 28$ females; experiment 2: $n = 12$ females) and Mainz, Germany (experiment 1: $n = 0$; experiment 2: $n = 18$). All these females and progeny were maintained under standard laboratory conditions (detailed in Meunier et al. 2012). Our 2 experiments started 5 days after the females' first clutch of eggs hatched and both involved isolated nymphs as feces consumers, as well as groups of nymphs and isolated mothers as feces producers. Five days after egg hatching, 5 (experiment 1) or 6 (experiment 2) nymphs per clutch were individually isolated in Petri dishes to be used later as potential feces consumers. Simultaneously, 2 groups of 7 nymphs (experiment 1) or all the remaining nymphs (experiment 2) and each mother (both experiments) were maintained in separate Petri dishes to produce the feces subsequently offered to the feces consumers. These groups of nymphs and mother donors immediately received an *ad libitum* amount of standard food (experiment 1) or pollen pellets (Hochland Bio-Blütenpollen; experiment 2), both dyed with blue food dye (Deko Back, Reichartshausen, Germany). Pollen pellets were standardized in size and shape using a metal punch press. The use of food dye increased the visibility of feces for the experimenter, but does not affect other feces properties (Diehl et al. 2015). The standard food was lab-made and mainly included pollen, carrots, cat food, and agar (see details in Kramer et al. 2015). All Petri dishes were 5.5 cm in diameter and were furnished either with moist sand (experiment 1) or with a circular sheet of filter paper (Macherey-Nagel GmbH & Co. KG, Düren, Germany) replaced for every test (experiment 2) and used to further increase feces visibility.

Experiment 1: feces, food deprivation, and nymph survival

In this first experiment, we aimed at testing whether access to feces improves nymph survival, and whether this effect depends on the presence of regular food. One day after isolation, each of the 5 nymphs per clutch were weighed to the nearest 0.001 mg using a microscale (model MYA5; PESCALE, Bisingen, Germany) and then haphazardly attributed to one of the 5 following treatments. Nymphs received either 1) standard food, 2) feces produced by 7 of their siblings over the previous days, 3) feces produced by their own mother over the previous days, 4) standard food plus feces produced by 7 of their siblings over the previous days, or 5) nothing. The treatments were renewed every 3 days using freshly produced feces and/or standardized food for a total of 25 days, during which we recorded nymph survival. Note that the amount of food/feces provided was the same across treatments and corresponded to the total amount of feces produced by the group of donor nymphs over the 3 previous days, while food was provided *ad libitum*. Note that the feces provided were never depleted by the focal nymph over

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3 days. To facilitate feces manipulation, each treatment was applied by transferring the isolated nymph into a Petri dish that was formerly occupied by the corresponding group of nymphs or was outfitted with either mother feces or a food source.

Experiment 2: food choice

In the second experiment, we tested whether nymphs show feeding preferences between feces and standard food, between feces of mothers and nymphs, or between feces of related and unrelated individuals. Note that here, relatedness is confounded with familiarity. Five days after their isolation, each of the 6 nymphs per clutch were haphazardly assigned to one of the 6 following food-choice setups: 1) food and feces of their own mother, 2) food and feces of their sibling nymphs, 3) feces of sibling nymphs and unrelated nymphs, 4) feces of their own mother and an unrelated mother, 5) feces of their own mother and sibling nymphs, or 6) feces of an unrelated mother and unrelated nymphs. We used the total amount of feces produced by the group of remaining nymphs and/or by the mothers during the 4 days preceding the tests. The feces and food source provided during the experiments only covered a small fraction of each experimental arena (about 2–3 mm^2) and were always provided in a quantity larger than the tested individual could possibly consume. For each test, the substrates were deposited on 2 opposite sides of the Petri dishes (changed every trial) using a clean metal stick. The focal nymph was placed in the center of the arena using soft steel forceps. We then recorded the time each nymph spent on each type of substrate over 30 min using a Sony HDR-CX200E video camera (movies started 5 min after setup to allow the nymphs to acclimate to the environment). Because earwigs are nocturnal, all filming was done under red light. To confirm that the time nymphs spent chewing on the substrate reflected food consumption, we also weighed a random subset of 70 nymphs originating from 12 clutches (Mainz population) before and after the tests and correlated this weight change to the time spent chewing the substrates. All videos were analyzed blindly regarding the origin of the assigned feces using the software "The Observer XT11" by Noldus.

Statistical analyses

All statistical analyses were performed using the statistics software R v3.0.3 (http://www.r-project.org/) loaded with the packages *survival*, *MASS*, and *car*. Nymph survival (experiment 1) was tested using a Cox proportional hazards regression model allowing for censored data, that is, nymphs alive at the end of the experiment. In this model, the nymph weight, the treatment (with 5 levels corresponding to the 5 tested treatments) and their interactions were entered as explanatory variables. To control for the non-independence of the nymphs used in this experiment (5 nymphs per clutch), the clutch of origin of each nymph was entered as a random effect into the model using the *frailty* argument. The significant effect of treatment (see Results for details) was further investigated using model estimates. To correct for multiple testing, the significance level for these pairwise analyses was adjusted using the MFDR (mean false discovery rate) approach to $\alpha_c = 0.033$ according to $\alpha_c = (n + 1)/(n \times 2) \times 0.05$, where n denotes the number of tests (Benjamini and Hochberg 1995). Note that all the individuals used in experiment 1 came from a single population.

The food-choice experiment was analyzed in 3 steps. In the first step, we conducted a series of 6 *t*-tests to determine whether the population of origin determined the proportion of time a nymph spent chewing one of the 2 assigned substrates (i.e., time chewing on substrate 1 divided by the total time spent chewing on both substrates combined). Because we found no population effect (Supplementary Table S1), we then pooled the populations per type of food-choice experiment and conducted a new series of 6 one-sample *t*-tests, with which we tested whether the proportion of time a nymph spent chewing on one of the 2 assigned substrates was significantly different from 0.5. In the last step, we used 2 one-sample *t*-tests to analyze whether nymphs still went to the feces side when they faced a choice between food and feces. Specifically, these analyses tested whether the proportion of time a nymph spent chewing on a food substrate was significantly different from 1. These 3 steps only included the trials during which nymphs were seen chewing on at least one of the 2 substrates. Finally, we investigated whether the time nymphs were seen on the substrates reflected an actual food intake, that is, if time spent on a substrate correlated with the absolute weight gained by nymphs during the test, using a linear regression model. We controlled for homoscedasticity and the Gaussian distribution of model residuals in each of these statistical analyses.

RESULTS

Experiment 1: feces, food deprivation, and nymph survival

Overall, 64.3% (90 of 140) of the isolated nymphs died during the 25 days of the experiment. This survival rate depended on the type of feces provided to the nymphs, as well as on the presence of an additional food source (Figure 1; Treatment effect: Likelihood Ratio [LR] $\chi^2_4 = 98.70$, $P < 0.0001$). Specifically, nymphs with access to nymphal feces survived longer than nymphs with access to maternal feces and longer than nymphs that had access to neither feces nor food source (Table 1). Moreover, the nymphs with access to an additional food source (with or without nymphal feces) survived longer than the nymphs with access to feces only (Table 1). By contrast, there was no difference in survival rates between the nymphs that had access to maternal feces and the ones that had access to neither feces nor a food source (Table 1). There was also no difference in the survival rates of nymphs provided with regular food or with regular food plus nymph feces (Table 1). Finally, heavy nymphs survived longer than light ones (LR $\chi^2_1 = 9.66$, $P = 0.002$; model estimate ± standard error [SE] = 4797.7 ± 1772.4), an effect that was independent of the treatment (interaction between nymph weight and treatment; LR $\chi^2_4 = -0.24$, $P = 0.999$).

Experiment 2: food choice

The proportion of nymphs that visited and chewed on at least one of the 2 assigned substrates did not differ between treatments (Fisher exact test, $P = 0.699$). Across the 30 videos recorded per treatment, nymphs visited at least 1 food source in 25 (83%) of the tests offering regular food and mother feces, 29 (97%) offering regular food and nymph feces, 27 (90%) offering related and unrelated mother feces, 26 (87%) offering related and unrelated nymphs feces, 26 (87%) offering related mother and nymph feces, and finally 27 (90%) offering unrelated mother and nymph feces. In these tests, nymphs spent more time chewing on regular food than on feces produced by related mothers (Table 2a) or feces produced by related nymphs (Table 2b; Figure 2). Nevertheless, during these 2 food-choice tests, the nymphs still spent a significant amount of time chewing on feces (one-sample *t*-tests against 1; Mother feces: $t_{24} = -2.69$, $P = 0.0127$; Nymph feces: $t_{28} = -4.30$, $P = 0.0002$). In the other tests, nymphs did not display a difference in time spent chewing on feces produced by either related and unrelated mothers

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(Table 2c) or by related and unrelated nymphs (Table 2d; Figure 2). Finally, they did not discriminate between the feces produced by related mothers and related nymphs (Table 2e), but spent more time on mother than nymph feces when both were produced by unrelated individuals (Table 2f; Figure 2). Based on the 70 nymphs that had been weighed before and after the food-choice test, we found a positive association between the total time spent chewing the substrates and the absolute weight gained by nymphs (LM, Model estimate ± SE = 0.0003 ± 0.0002, $t = 2.65$, $P = 0.010$).

DISCUSSION

The accumulation of feces in a nest is often associated with detrimental effects, such as pathogen spread and growth and attraction of parasites, all of which could ultimately hamper the evolution of group living (Vet and Dicke 1992; Schmid-Hempel 1998; Steidle and Fischer 2000; Sato et al. 2003; Sagara et al. 2006; Weiss 2006). Yet, a few species such as the European earwig still maintain feces in their nest and even consume feces produced by group members (Nalepa et al. 2001; Rosengaus et al. 2013; Falk et al. 2014; Diehl et al. 2015; Kramer et al. 2015; Kramer and Meunier 2016). Here, we aimed to improve our understanding of the evolution and maintenance of this behavior in earwigs by investigating the benefits of and the mechanisms regulating allo-coprophagy. Our results demonstrate that access to sibling feces enhanced the survival rate of nymphs in the absence of a regular food source. However, this effect was absent when nymphs had access to maternal feces and disappeared when they also had access to a regular food source. In this experiment, we also showed that nymph weight positively affected their survival, independently of their access to feces and/or a food source. Our food-choice experiments then showed that nymphs always preferred the consumption of food over feces, even if feces consumption still occurred during the tests. These experiments also revealed that nymphs did not discriminate between feces from related and unrelated individuals or between maternal or nymphal feces produced by related individuals. However, when offered the choice between feces produced by unrelated mothers and nymphs, they preferred the former.

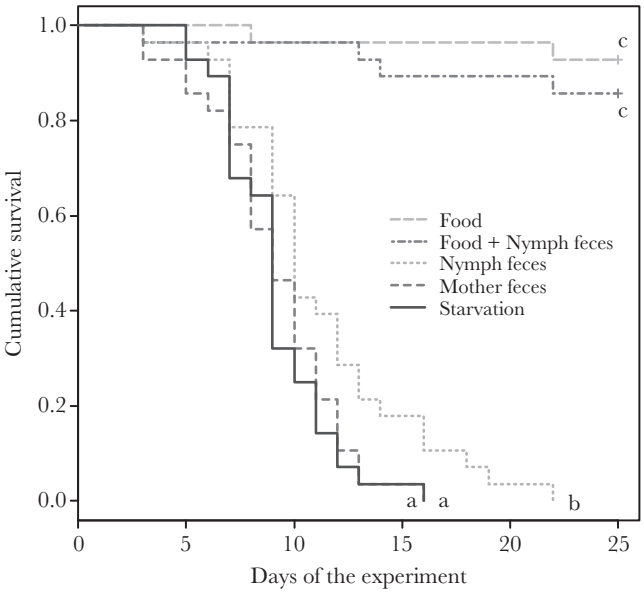

Figure 1
Influence of food and feces access on nymph survival. Individual lines represent the cumulative survival rate of nymph that had access to either standard food (median Lethal Time [LT50], LT_{50} ± SE = 49.8 ± 7.7), feces produced by their siblings (LT_{50} = 15.6 ± 0.3), feces produced by their own mother (LT_{50} = 40.7 ± 3.4), standard food plus feces produced by their siblings (LT_{50} = 15.6 ± 0.3), or nothing (LT_{50} = 13.5 ± 0.2). Different letters indicate P value <0.03 (see Table 1).

Our data demonstrate that the consumption of nymphal feces significantly delayed death by starvation, a result in line with a nutritional benefit of (allo-)coprophagy. Intraspecific coprophagy for the sake of nutrient intake is well documented in many different taxa including rodents, insects, gastropods, and amphibians (Steinwascher 1978; Stevenson and Dindal 1987; Brendelberger 1997; Takahashi and Sakaguchi 1998; Nalepa et al. 2001). Interestingly, in earwigs, the benefits of coprophagy only occurred when nymphal feces were consumed, while consumption of maternal feces yielded no such effect. On a proximate level, this result may reflect an age-specific efficiency of the digestive tract in earwigs. The stability of the hindgut fauna is indeed known to become increasingly efficient during insect development (Engel and Moran 2013), so that feces produced by adults are less likely to contain a large concentration of undigested particles (in other words, they are more likely to exhibit a high nutritional value). Alternatively, this age-specific benefit on nymph survival could rely on differences in the occurrence and/or proportions of various microbial proteins and other components of the hindgut fauna that exhibit specific nutritional values. The consumption of such components through coprophagy is indeed known to provide nutritional benefits in other insect species (Nalepa et al. 2001) and their age-specific variation could thus also explain a delayed death by starvation in nymphs. The efficiency of juveniles and adult digestive tracts, as well as the composition of their hindgut fauna, however, remains to be studied in earwigs.

Despite a general preference for regular food compared to feces, we showed that nymphs always consumed some (maternal or nymphal) feces when they also had access to regular food.

Table 1
Pairwise differences between treatments in the survival experiment (experiment 1)

	Food	Food + nymph feces	Nymphs feces	Mother feces	Starvation
Food		χ^2_1 = 0.73	χ^2_1 = 30.21	χ^2_1 = 37.77	χ^2_1 = 39.21
Food + nymph feces	P = 0.390		χ^2_1 = 35.32	χ^2_1 = 44.92	χ^2_1 = 46.94
Nymphs feces	**P < 0.0001**	**P < 0.0001**		χ^2_1 = 5.02	χ^2_1 = 6.28
Mother feces	**P < 0.0001**	**P < 0.0001**	**P = 0.025**		χ^2_1 = 12.2
Starvation	**P < 0.0001**	**P < 0.0001**	**P = 0.012**	P = 0.140	

The reported values are obtained from Coxph model estimates. P values still significant after MFDR correction are in bold.

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This finding indicates that the incentives for coprophagy in earwigs are not limited to the acquirement of nutrients and their aid against starvation. A well-documented mediator of non-nutritional benefits of coprophagy is the transfer of microbes, such as the ones constituting the mutualistic hindgut fauna of an animal (Dillon and Dillon 2004; Weiss 2006; Engel and Moran 2013). In vertebrates, the acquisition of this hindgut fauna through coprophagy has been shown to be of high importance for the development of juveniles in terms of growth and body size, such as in the rat *Rattus norvegicus* (Fitzgerald et al. 1964) and tadpoles of the American bullfrog *Rana catesbeiana* (Steinwascher 1978). Similarly, coprophagy and (primarily) proctodeal trophallaxis are essential for survival in many xylophagous insects, as it mediates the distribution of crucial cellulose-digesting gut symbionts (Cleveland 1925). In termites, repeated coprophagy and proctodeal trophallaxis are of special importance because a majority of gut protists is lost during molting and reacquired through coprophagy (Nalepa 2015). The transfer of immune and/or antimicrobial components could be another, though non-mutually exclusive, non-nutritional benefit for coprophagy. In earwigs, a recent study revealed that feces exhibit antimicrobial properties and that these properties depend on whether it was produced by nymphs or mothers (Diehl et al. 2015). However, whether individuals can acquire these immune components through allo-coprophagy remains to be investigated in earwigs and in insects in general (see Mirabito and Rosengaus 2016).

Table 2

Differences in the proportion of time spent by nymphs on each type of substrate in the 6 types of food choice

Food-choice test	*t*-value	df	*P* value
(a) Regular food vs. related mother feces	6.14	24	**<0.0001**
(b) Regular food vs. related nymph feces	3.49	28	**0.002**
(c) Related mother feces vs. unrelated mother feces	−1.31	26	0.202
(d) Related nymph feces vs. unrelated nymph feces	1.04	25	0.309
(e) Related mother feces vs. related nymph feces	1.12	25	0.272
(f) Unrelated mother feces vs. unrelated nymph feces	3.10	26	**0.005**

Significant *P* values are in bold.

Our second experiment also revealed that nymphs did not exhibit a preference for the consumption of feces produced by related as compared to unrelated individuals. This suggests that the nutritive and/or non-nutritive benefits of allo-coprophagy do not require a genetic similarity (or familiarity) between donors and recipients. It is nevertheless important to note that this effect might also reflect the fact that, even if clutch joining occurs under natural conditions (Kölliker and Vancassel 2007), nymphs mostly encounter feces produced by related individuals within the nest and might therefore not have been able to evolve such a discrimination capability. More generally, further studies should investigate why nymphs exhibited a preference for maternal over nymphal feces when these producers were all unrelated to the consumer.

Across species and taxa, the benefits of family life are typically mediated by 1 or 2 caring parents (Trivers 1972; Royle et al. 2014), while siblings are often thought to only fight and compete over the distribution of resources (Mock and Parker 1997; Roulin and Dreiss 2012). However, an increasing number of studies demonstrate that sibling interactions can be mutually beneficial despite the high potential for competition (Roulin et al. 2012; Kramer and Meunier 2016; Roulin et al. 2016). In earwigs, previous studies demonstrated that nymphs not only share food via allo-coprophagy, but also increase feces production when in contact with related as compared to unrelated nymphs (Falk et al. 2014). Here, we demonstrate that this behavior is (at least partly) a means of helping clutch members to survive under food deprivation and also suggest that it could mediate the exchange of microbial fauna between family members. Interestingly, sanitary behavior (such as a feces removal) has been considered to be an important facilitator for the evolution of eusociality (Jackson and Hart 2009). However, in the early stages of social evolution, such as found in species with facultative family

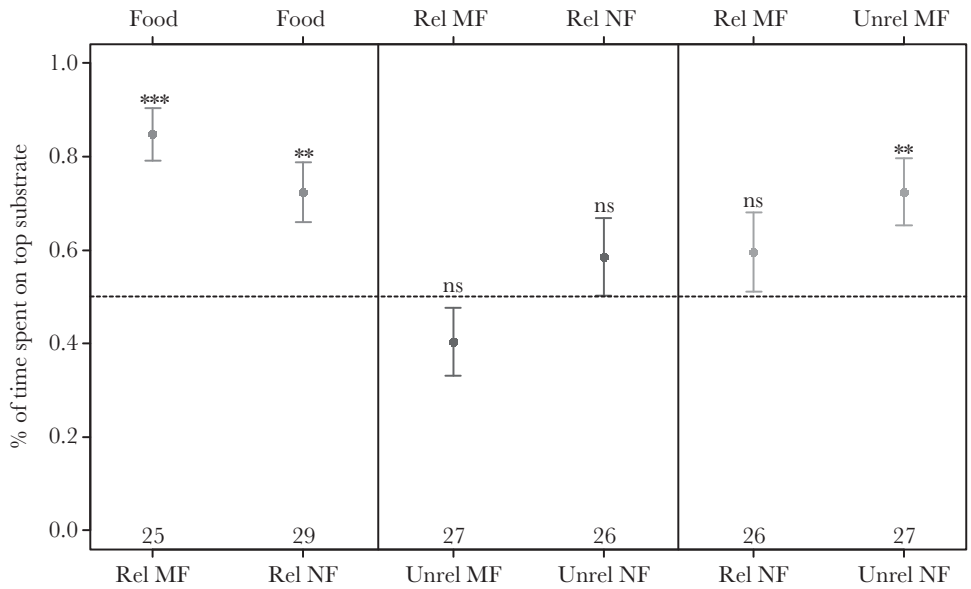

Figure 2

Proportion of time spent by nymphs on the top—compared to the bottom—reported type of substrate in the 6 food-choice trials. The food choices involved regular food (Food), as well as mother feces (MF) and nymph feces (NF) produced by related (Rel) and unrelated (Unrel) individuals. The number of nymphs that visited one of the 2 substrates at least once is reported at the bottom of each line. The mean ± standard error is reported. ***$P < 0.001$, **$P < 0.01$, ns = not significant.

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life, the group is maintained for a relatively short time period, so that sanitation may not be a necessity if the nest site can be abandoned before the adverse effects set in. In such a scenario, beneficial effects of feces exchange, such as shared resources and hindgut fauna, could thus be selected for and ultimately promote cooperative behaviors that delay group dispersal and thereby drive the evolution of sociality.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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Review

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Social immunity and the evolution of group living in insects

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The evolution of group living requires that individuals limit the inherent risks of parasite infection. To this end, group living insects have developed a unique capability of mounting collective anti-parasite defences, such as allogrooming and corpse removal from the nest. Over the last 20 years, this phenomenon (called social immunity) was mostly studied in eusocial insects, with results emphasizing its importance in derived social systems. However, the role of social immunity in the early evolution of group living remains unclear. Here, I investigate this topic by first presenting the definitions of social immunity and discussing their applications across social systems. I then provide an up-to-date appraisal of the collective and individual mechanisms of social immunity described in eusocial insects and show that they have counterparts in non-eusocial species and even solitary species. Finally, I review evidence demonstrating that the increased risks of parasite infection in group living species may both decrease and increase the level of personal immunity, and discuss how the expression of social immunity could drive these opposite effects. By highlighting similarities and differences of social immunity across social systems, this review emphasizes the potential importance of this phenomenon in the early evolution of the multiple forms of group living in insects.

1. Introduction

The shift from a solitary life to group living is considered to be one of the major evolutionary transitions [1,2]. To date, group living can be found in almost all animal taxa, with its complexity ranging from simple mutual attraction between individuals, over temporary periods of parental care in family associations, to permanent societies with reproductive division of labour [3,4]. The ecological success of group living species is traditionally thought to rely on the fitness benefits social life provides to group members, such as improved protection against predators, increased reproductive success, enhanced foraging efficiency and higher survival rates [5]. However, group living also comes with major fitness costs. One of these costs is the increased risks of parasite infection (broadly defined to include bacteria, viruses, protozoans, helminths and fungi) [6–12]. These risks are particularly exacerbated in group living species, because frequent and intimate contacts between individuals are known to facilitate parasite transmission, and because the close genetic relatedness often exhibited by group members is likely to make them susceptible to the same parasites [6,7,13–15]. Hence, to gain a better understanding of the evolution of group living, we need to shed light on the mechanisms that limit the inherent risks of parasite infections for group living individuals [7].

Group living individuals are not only able to limit parasite infection using their personal immune system [16,17], but may also use their unique capability of mounting collective defences. This phenomenon, called social immunity (see below for a more detailed definition [6,7,18]), encompasses multiple mechanisms such as the use of antimicrobial substances as nest material and sanitation behaviours to eliminate corpses and waste products from the nest (figure 1). Over the last 20 years, eusocial insects, which mostly include ants, termites, as well as some bees and wasps, have provided one of the main biological models to study social immunity [7,19–21]. The resulting studies were of key importance, because they revealed the structural diversity of social immunity across eusocial species and

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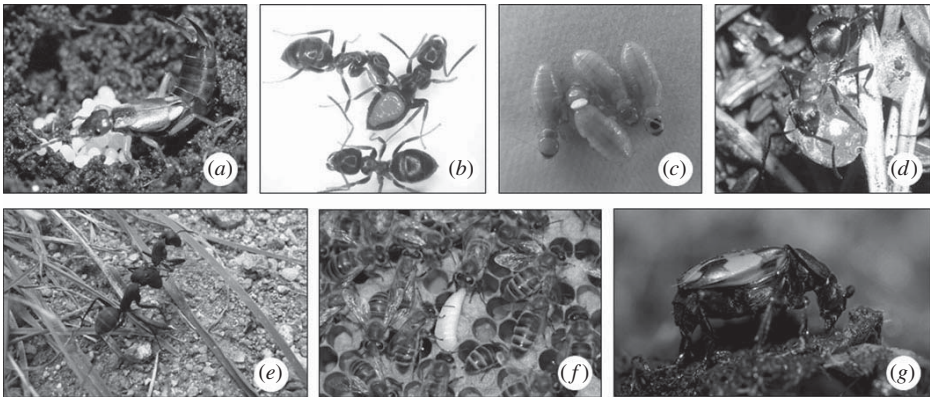

Figure 1. Examples of social immunity in insects. (*a*) A female of the European earwig *Forficula auricularia* cleaning her eggs from fungal spores (Picture credit: J. Meunier). (*b*) Workers of the invasive garden ant *Lasius neglectus* allogrooming a colour-marked nest-mate exposed to an entomopathogenic fungus (Picture credit: M. Konrad, IST Austria). (*c*) In the dampwood termite *Zootermopsis angusticollis*, workers express alarm behaviours to limit the spread of pathogens among colony members (Picture credit: R. Rosengaus). (*d*) A worker of the wood ant *Formica paralugrubris* using resin as nest material to enhance protection against parasite infections (Picture credit: A. Maeder). (*e*) A worker of the ant *Camponotus* sp. carrying a corpse away from the nest (Picture credit: L. Diez). (*f*) Honeybee workers removing diseased larvae from an uncapped cell (Picture credit: G. Bigio). (*g*) A female of the burying beetle *Nicrophorus vespilloides* using the antimicrobial properties of her anal exudates to protect the nest against parasites (Picture credit: O. Krueger). (Online version in colour.)

provided clear evidence for the importance of this phenomenon in permanent and complex social groups [7,19]. However, these studies were of limited relevance for our understanding of the role of social immunity in the early evolution of social life. In particular, it remains unclear whether social immunity only emerged in eusocial systems and therefore represents a secondary trait derived from eusociality, or whether it has counterparts in less derived forms of group living and thus possibly plays a role in the emergence and maintenance of group living.

The aim of this review is to explore whether social immunity is a driver or a by-product of the evolution of complex forms of group living in insects. This review focuses on insects, because they exhibit the greatest diversity of social systems across animal taxa [3,4], including most of the eusocial species currently known, and because they represent a majority of the studies in which social immunity has been studied [7,15,22]. I begin by presenting different definitions of social immunity found in the literature and discuss their importance to study this phenomenon across social systems. I then provide an up-to-date appraisal of the collective and individual anti-parasite mechanisms reported in eusocial insects and, where applicable, link these mechanisms to their known counterparts in non-eusocial and solitary species [23]. Non-eusocial species are defined as all species in which group living is temporary or permanent, facultative or obligatory, but where it is not associated with reproductive division of labour (for a full description of the multiple forms of group living, see [3,4]). In a third part, I review empirical evidence showing the positive and negative influence of group living on the expression of personal immunity and discuss the role of social immunity on the nature of these effects. Finally, I discuss how currently available data provide insights on the possible role of social immunity in the evolution of group living, but also emphasize the need to study social immunity in a larger number of non-eusocial insects.

2. Definitions of social immunity

The first definition of social immunity in an evolutionary context was offered in a landmark paper published in 2007 by Cremer *et al.* [7]. They defined social immunity as 'collective action or altruistic behaviours resulting in avoidance, control or elimination of parasitic infections'. This definition, followed by the shorter one 'collective defences against parasites and pathogens' by Wilson-Rich *et al.* in 2009 [19], was coined to describe the group-level defences against parasites expressed in colonies of eusocial insects (and to some extent in groups of primates). These defences included allogrooming, a behaviour during which one individual grooms another individual to remove its external parasites, and hygienic behaviours, during which diseased brood are removed from the nest [7,19] (see also figure 1).

In an important review published in 2010, Cotter & Kilner [23] proposed to broaden the field of social immunity by applying the theory previously developed in the context of eusocial insects to other forms of group living. To this end, social immunity was defined as 'any type of immune response that has been selected to increase the fitness of the challenged individual and one or more recipients'. One major benefit of defining social immunity in a broader context than eusociality was to allow the inclusion of anti-parasite defences that are not tightly linked to permanent social organization, such as parental care during family life (e.g. [24]) and to some extent herd immunity [25] (see also conclusion in [7]). Another benefit of the definition by Cotter *et al.* was that it clarified that social immunity refers to defences that have been selected for their collective anti-parasite protection, and not the ones that provide benefits to group members as a simple by-product of personal protection. This distinction is of key importance, because social immunity could otherwise encompass the strategies used by solitary-living individuals to fight their own infection, as these strategies somewhat reduce parasite exposure of the conspecifics they might encounter during their life cycle (e.g. during mating or competitive interactions).

The definition proposed by Cotter & Kilner [23] also raised two potential issues. First, it frees the recipients from being a member of the donors' group, so that social immunity might not be a product of social life, but simply the outcome of shared location between individuals (even from different species) [23]. For instance, this definition allowed social

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immunity to encompass symbionts protecting their hosts, which is unlikely to reflect social life. The second potential issue comes from the strong emphasis on the fitness benefits for both donor and recipient(s). This may suggest that no cost is involved in social immunity, which in addition to being an unlikely scenario (e.g. [26]), would also raise the question of why not all anti-parasitic defences have evolved towards this universally beneficial state.

To circumvent the issues raised by the definition of Cotter & Kilner [23] while adopting their proposal to expand the field of social immunity beyond eusociality, I propose to define social immunity as 'any collective and personal mechanism that has emerged and/or is maintained at least partly due to the anti-parasite defence it provides to other group members'. Readers interested in details of terminologies covering individual and social immunization are referred to the review by Masri & Cremer [22].

3. Social immunity in eusocial, non-eusocial and solitary insects

Infecting multiple members of an insect group generally requires that parasites successively pass three steps [7,19]. The first one consists of the uptake of the parasite from the environment, the second step is its successful transfer into the nest and the third one is its multiplication and transmission to other group members. Any individual or collective strategy reducing the success of parasites in at least one of these three steps thus possibly qualifies as a mediator of social immunity (given that its collective benefits are under selection). In their seminal study, Cremer *et al.* [7] reviewed the large diversity of anti-parasite mechanisms employed by eusocial insects to diminish the risks associated with each of the three steps of infection. Some of these mechanisms require traits that are specific to eusocial systems, such as division of labour, so that their expression is unlikely to exist in other forms of group living. However, many of them do not have such requirements, which offers scope for their expression in non-eusocial species, and even to find counterparts in solitary species [23].

In the following sections, I provide an up-to-date appraisal of the anti-parasite mechanisms involved in each of the three steps of group infection in eusocial insects (adapted from Cremer *et al.* [7]) and then determine whether they have known counterparts in non-eusocial and solitary insects. I summarize what is known in table 1, which is organized around the three steps to infection just described. Based on this information, I then discuss potential reasons for the presence/absence of these defences across the different forms of group living.

(a) Step 1: limiting parasite uptake from the environment

The most common strategies used by eusocial insects to limit parasite uptake from the environment encompass both collective and individual mechanisms that help to avoid infection after or during foraging activities. Collective defences are illustrated, for instance, by guards of the honeybee *Apis mellifera*, which prevent newly infected nest-mates from entering the hive [38] and 'hitchhikers' of leaf-cutter ants, which ride on leaf fragments to defend foragers against parasitoid attacks [37]. On the other hand, individual defences can be exhibited by foragers that avoid habitats containing entomopathogenic fungi and nematodes, such as in the termite *Macrotermes michaelseni* and the ant *Solenopsis invicta* [27,28], respectively, as well as by other ant species that refrain from the consumption of contaminated food or conspecifics [32,33].

The individual anti-parasite mechanisms reflected by avoidance behaviours are not specific to eusocial insects and can be found in many non-eusocial and solitary species. For example, gregarious individuals of the migratory grasshopper *Melanoplus sanguinipes* and the German cockroach *Blattella germanica* avoid the consumption of contaminated food/conspecifics [34,35]. In the subsocial burying beetle *Nicrophorus vespilloides*, females avoid degraded carcasses (covered with many microbes) if given a choice with fresh ones, a mechanism that could also have evolved to reduce post-hatching competition between juveniles and microbes over the carcass [30]. Finally, in the solitary-living Japanese beetle *Popillia japonica* and the seven-spot ladybird *Coccinella septempunctata*, individuals avoid soil contaminated with entomopathogenic fungi [31] and larvae inhibit their consumption of infected prey [36], respectively. Because avoidance behaviours provide direct fitness benefits to the actors, the importance of social interactions in the emergence and/or persistence of these behaviours in social species, and thus their qualification as social immunity, remains to be further explored [7].

(b) Step 2: limiting parasite establishment into the nesting habitat

Incorporating chemical/antimicrobial substances into the nest material and/or expressing nest sanitation behaviours are the two main processes reported in eusocial insects to prevent parasite establishment in their habitat. Chemical substances can be collected from the environment, such as in the wood ant *Formica paralugubris* and the honeybee in which workers collect pieces of resin and incorporate them into nest structure (figure 1, [39,40]), or they may be synthesized by colony members through metapleural, sternal or venom glands [41–43,83] (reviewed in [84]). Sanitation behaviours involve eliminating waste material, such as conspecific corpses, left-over food or frass that could be used as a substrate by the parasites [54,55,85].

The use of chemical/antimicrobial substances and sanitation behaviours can also be found in many non-eusocial species [84]. For instance, adults of the group living beetle *Dendroctonus rufipennis* exude oral secretions in their galleries to inhibit the growth of colonizing fungi [44]. Subsocial females of the beewolf *Philanthus triangulum* cultivate *Streptomyces* bacteria on their antenna and apply them to brood cells to protect cocoons from fungal infection [45]. Frass removal occurs in the subsocial cockroach *Cryptocercus punctulatus* [57], the group living beetle *Trachyostus ghanaensis* [58] and the subsocial cricket *Anurogryllus muticus* [59]. Frass removal can even be found in solitary insects exhibiting high site fidelity, such as the grasshopper *Atractomorpha lata* and in *Epargyreus clarus* caterpillars, in which frass pellets are ballistically ejected by the producing individuals [56,60].

Interestingly, an alternative strategy to frass removal is to imbue frass or anal exudates with antimicrobial properties and use them in the nest to limit parasite establishment. This process occurs in both eusocial (termites) and non-eusocial (cockroaches and burying beetles) species, in which the use

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Table 1. Personal and collective mechanisms shaping social immunity in eusocial insects, and their counterparts in non-eusocial and solitary species. Requirements for their expression are listed for each mechanism. The mechanisms can be absent/not reported (?) or not expected to occur in the given system (x). Table adapted from Cremer *et al.* [7], which also contains information on whether these defences are prophylactic or induced. References [113–219] are given in the electronic supplementary material.

defence mechanisms	requirements	eusocial	non-eusocial (group living)	solitary
Step 1. Limiting parasite uptake from the environment				
only a small proportion of group members forage	stable habitat (e.g. nest) division of labour	[113]	x	x
avoidance of contaminated habitats (incl. oviposition places)		[27–29,114–116]	[30,117]	[31,118–120]
avoidance of consumption/contact with infected food/conspecifics (incl. cannibalism)		[32,33,121–124]	[34,35]	[36,119,120, 125–127]
guarding of foraging trails	stable habitat (e.g. nest) foraging trails division of labour	[37]	x	x
guarding of nest entrance	stable habitat (e.g. nest) division of labor	[38,128]	x	x
Step 2. Limiting parasite establishment into the nesting habitat				
collection of antimicrobial substances	stable habitat (e.g. nest)	[39,40,129, 130]	?	x
use of self-produced chemical secretions on habitat surface (e.g. metapleural or sternal glands, venom, other)	stable habitat (e.g. nest)	[20,41–43,131–134]	[44,45,135]	x
faecal material/anal exudates with antimicrobial properties cover the nest	stable habitat (e.g. nest)	[46–48]	[26,49–53, 136,137][a]	x
corpse removal	stable habitat (e.g. nest)	[54,138–144]	?	x
garbage and frass removal/separated waste dump	stable habitat (e.g. nest)	[55,56,145–150]	[56–59, 151–154]	[60][b] [56]
cover/encapsulate corpses or parasites within the nest	stable habitat (e.g. nest)	[33,143,155–157]	?	x
Step 3. Limiting parasite transmission between group members				
(*a*) From adults to brood				
mechanical removal by allogrooming (adults-to-brood)	parental/sibling care	[20,158]	[24,159,160]	x
application of antimicrobial secretion on the brood/the eggs	parental/sibling care	[61,161–163]	[24][c]	x
feed the brood with anti-parasitic components	parental/sibling care	[62,164–166]	[63,167–172]	x
infected individuals do not tend the queen/brood	communal breeding or precocial species	[64,65]	?	x
removal of diseased brood	parental/sibling care	[66,67,173,174]	?	x
trans-generational immune priming		[68,175]	[69]	[176–180][d]
(*b*) From adults to adults				
mechanical removal by allogrooming (adult-to-adult)	behavioural interactions	[20,33,64,70,71, 158,181–192]	[72][e]	x
social fever		[73,193]	[74][f]	[74][f]
spatial compartmentalization of the nest/digging area	stable habitat (e.g. nest) complex nest architecture	[13,113, 194–198]	?	x
infected individuals leave the group/limit contact to group members		[65,75,199,200]	?	x

(*Continued.*)

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Table 1. (*Continued.*)

defence mechanisms	requirements	eusocial	non-eusocial (group living)	solitary
behavioural structuring (castes/age)	overlapping generations or castes	[6,201,202]	x	x
indirect interaction with garbage workers	stable habitat (e.g. nest) division of labour	[145]	x	x
pathogen alarm		[71,76]	?	x
isolation of infected individuals	stable habitat (e.g. nest) complex nest	[29]	?	x
remove/cannibalize infected individuals/brood from the group		[66,67,77,158, 174,203–207]	?	x
abandon infected nests	stable habitat (e.g. nest)	[20,78,124]	?	x
immunity transfer by social interactions	behavioural interactions	[65,70,208]	?	x
(*c*) Genetic adaptations				
increased genetic diversity (multiple mating, multiple queens)	stable group composition	[66,79,80, 209–216]	but see [81]	x
increased recombination rates		[82,217,218]	?	x

[a]In burying beetles, this mechanism could help mothers and offspring to limit competition with microbes over the carcasses.
[b]Frass ejection.
[c]Application of cuticular hydrocarbons on the brood.
[d]Could also be considered as a form of parental care [219].
[e]Role against parasite infection still unclear.
[f]Behavioural fever.

of frass as nest material has been shown to limit parasite development [46,47,49–51]. The maintenance of frass in the nest may also provide immune benefits by allowing exchanges of endosymbionts and other immune factors among group members. For instance, in the bumblebee *Bombus terretris*, individuals obtain bacterial microbiota by feeding on conspecific faeces, which improves their resistance against the protozoan parasite *Crithidia bombi* [48]. To date, this latter benefit remains unexplored in non-eusocial insects.

(c) Step 3: limiting parasite transmission between group members

When a parasite has successfully passed the two first steps of group infection, the expression of mechanisms limiting its transmission among group members is required. Eusocial and non-eusocial species share most of the mechanisms known to limit the transmission of parasites from adults to offspring (including eggs and juveniles). This is expected as many group living species with non-eusocial systems are characterized by various forms of parental care, which possibly include anti-parasite defences [86]. These mechanisms include adults grooming the brood to remove external parasites [20,24], applying antimicrobial secretions to the brood [24,61], providing food with antimicrobial properties to the brood [62,63] or transferring immune components to their eggs (called trans-generational immune priming [68,69]). The mechanisms solely present in eusocial insects consist of workers eliminating infected brood (hygienic behaviour, [66,67]) or of infected individuals avoiding contact with the brood [64,65]. Nevertheless, these two mechanisms could be expected in subsocial (and precocial) species.

Limiting parasite spread between group members is not only important for the brood, but also for adults. In eusocial species, multiple processes are known to limit parasite spread among adults, such as building nest architecture with small chamber areas [13], abandoning infected nests [78], boosting personal immunity by social contacts [70] or consuming infected group members [77] (table 1). Individuals of the dampwood termite *Zootermopsiss angusticollis* also express pathogen alarm behaviours when encountering infected conspecifics [71] (but also infected environments [76]), a behaviour that prevents pathogen spread among individuals by triggering avoidance behaviours. Infected individuals may also be isolated from the group either through exclusion, such as in the subterranean termite *Reticulitermes tibialis* [29], or through self-driven desertion (due to their moribund state), as in the ant *Temnothorax unifasciatus* [75]. Although the above processes do not necessarily require traits that are specific to eusocial insects (table 1), only allogrooming has been found in non-eusocial and/or solitary species. This behaviour is ubiquitous in eusocial insects [87], in which it reduces the presence of external parasites on the receiving individual, as well as improving the immune response of the donor through low dose infection [70]. Allogrooming has also been reported in non-eusocial insects, such as earwigs and cockroaches [72,88], but its importance in reducing parasite infection remains unclear. Note that the mediator of social fever is also not specific to eusocial systems. Social fever is a collective defence during which group members adaptively and temporarily increase their

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own temperature (behavioural fever) to increase nest temperature (then called social fever) to a level that is detrimental for parasites. This collective defence has only been reported in honeybees [73], but behavioural fever is a common anti-parasite defence in non-eusocial and solitary species (reviewed in [74]). Whether behavioural fever can be elevated to social fever in non-eusocial insects remains unknown.

Finally, the increase in genetic diversity within a group is also known to help prophylactically in reducing the risk of parasite spread between group members, including both adults and juveniles [89,90]. In eusocial insects, this resistance can be achieved by increasing the number of queens and/or of the queen's mating partners, as revealed by the higher survival rates of ant and honeybee workers living in infected colonies headed by multiple compared to single queens [79] or by multiply compared to singly mated queens [80], respectively. The benefits of increased genetic diversity have also been proposed to result from genomic recombination, which occurs at high rates in several eusocial species [82] (but see [91]). Somewhat surprisingly, the importance of group genetic diversity for parasite resistance remains poorly studied in non-eusocial insects. To the best of my knowledge, only a single study (indirectly) tested this effect in a gregarious insect, caterpillars of the moth *Malacosoma californicum pluviale*, but reported inconclusive results [81].

(d) Ubiquity of collective immunity across group living insects?

Although several mechanisms of social immunity are common to eusocial and non-eusocial species, others are unexpectedly absent in non-eusocial insects living in nests (e.g. during family life). This is the case, for instance, for corpse removal from the nest, abandoning of infected nests, increase in group genetic diversity to prophylactically resist against parasite infection, isolation of infected individuals or group desertion by infected individuals (table 1). One potential explanation for this lack of evidence is the very limited number of studies investigating social immunity in non-eusocial insects, even if a growing number of studies recently have started to fill this gap (e.g. [24,52,53]). Another possible explanation is that the emergence of these mechanisms depends on traits that are specific to eusocial insects, but not directly associated with their social system. For instance, colonies of eusocial insects are mostly composed of non-reproductive individuals, for which social immunity is the only way to protect the queen's brood and thus to favour their own (indirect) fitness under parasite attack [19]. Similarly, eusocial colonies may contain a large number of individuals, which could increase the efficiency and thus the use of collective anti-parasite defences compared with smaller groups [19]. Further research should thus be conducted to (i) enlarge the number of studies testing the expression of social immunity in non-eusocial species and to (ii) determine the importance of eusocial-derived traits on the emergence and expression of these collective defences.

Even if many studies reveal the occurrence of social immunity in eusocial species, it is important to note that others have failed at providing relevant support. For instance, queens of the wood ant *F. paralugubris* are specifically attracted to (instead of repelled from) habitats contaminated by entomopathogenic fungi [92]. Similarly in the pharaoh ant *Monomorium pharaonis*, colonies preferentially move into infected nests when presented with the choice between an infected and an uninfected one [93]. Finally, workers of the termite *Z. angusticollis* do not discriminate between infected and non-infected conspecifics [94], and parasite infection does not increase the expression of allogrooming in the ant *Formica selysi* [95]. Overall, these results provide important insights into the link between group living and social immunity because they (i) reveal that not all the mechanisms of social immunity are necessarily expressed during group living and (ii) shed light on the potential role of other factors, e.g. parasitic strategies and life-history costs, in the expression of collective defences. Furthermore, these results suggest that social immunity is not always the most efficient defence against parasite infection, which raises questions about the importance of personal immunity in group living individuals.

4. Personal immunity and group living

The early evolution of group living and its associated risks of parasite infection may not only have favoured the emergence of social immunity, but may also have selected for higher effort in personal immune responses. This higher effort would help group living individuals both to prophylactically limit a greater *per capita* risk of infection (*density-dependent prophylaxis* hypothesis, called DDP [96]) and to benefit from herd immunity, which is generated by the accumulation of resistant individuals within a group [97]. A series of three studies recently provided empirical support for DDP's prediction of a positive association between group living and efforts in personal immunity [98–100]. In these studies, authors compared the level of personal immunity across six species of bees, nine species of wasps and six species of thrips exhibiting a gradient of sociality ranging from solitary to eusocial. Each of these three studies showed that the antimicrobial secretions present on individuals' cuticles were significantly more efficient in eusocial compared with solitary species. Empirical support for this prediction also comes from within-species comparisons. For instance, in the desert locust *Schistocerca gregaria*, individuals in the gregarious phase showed higher antimicrobial activities and survived infections better than individuals in the solitary phase [101] (see other examples in [96]).

By contrast, it has been proposed that the emergence of social immunity could reduce the expression of personal immunity due to investment trade-offs between these two processes [7,23,90]. Three genomic and two physiological studies conducted with eusocial and non-eusocial insects supported this prediction. The genomic analyses showed that (i) the number of gene families implicated in immunity was three times lower in honeybees than in two solitary insects [90,102], that (ii) experimentally isolated *Bombus terrestris* workers up-regulated the expression of immune-related genes [103] and that (iii) the number of immune-related genes expressed in infected *S. gregaria* individuals was higher when they lived in the solitary compared with gregarious phase [104]. Moreover, the physiological studies revealed that across 12 Lepidoptera species, the caterpillars feeding solitarily had higher levels of personal immunity than the ones feeding gregariously [8]. Finally, in the Australian plague locusts *Chortoicetes terminifera*, the level of personal immunity was negatively correlated with population densities, while experimental isolation of individuals marching in bands yielded an increase in their personal immune levels [105].

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The discrepancy between these positive and negative associations sheds light on the two main assumptions required to expect a negative effect of group living on personal immunity. The first one is that group living is associated with the expression of social immunity, which is not necessarily the case in all group living insects (see above). The second one is that mounting a collective immune response is costly for the producers. To the best of my knowledge, only one study investigated this cost in a (sub)social insect. In particular, Cotter *et al.* [26] showed in the burying beetle *N. vespilloides* that increased investment in wound repair (personal immunity) caused a temporary decrease in the antibacterial activity of anal exudates (social immunity) of mothers. These exudates are used by the parents to limit microbial development on the carcass hosting their developing offspring [30]. This important finding raises questions about the general costs of social immunity across social systems and thus of its importance in the expression of personal immunity. Both questions call for further studies addressing this issue in a large number of eusocial and non-eusocial species. It is important to note, however, that the expression of such costs may depend on the intrinsic quality of the individuals, so that high-quality individuals could afford relatively high investment into both personal and social immune responses [106]. As a result, any variation in individual quality within a population could have the potential to prevent detection of the trade-off between the two types of immunity, or even to transform it into a false-positive association at the population (or species) level [107]. Future studies on the costs of mounting forms of collective immunity should therefore control for individual quality, e.g. by experimentally upregulating the social or personal immune response of a given individual and then looking for downregulation in other areas (e.g. [26]).

5. Discussion

Better understanding of whether social immunity is a driver or a by-product of the evolution of complex forms of group living first requires data on its occurrence across social systems [23]. This review reveals that out of the 30 individual and collective mechanisms known to mediate social immunity in eusocial insects and listed in table 1, 10 have counterparts in non-eusocial and four in solitary species. The distribution of these mechanisms across social systems is compatible with a scenario placing social immunity as a prerequisite for increased complexity in the nature and frequency of social interactions (i.e. in the evolution of group living). In particular, eusocial insects express the individual mechanisms used by solitary species to limit parasite uptake from the environment (first step of parasite infection), to which they add the collective ones used by non-eusocial species to limit both parasite establishment in the nest (second step of parasite infection) and parasite transmission to the brood (first part of the third step of parasite infection), as well as specific ones that limit parasite spread between adult group members (second part of the third step of parasite infection). Note, however, that this scenario does not exclude that (at least) some collective mechanisms are secondarily evolved in eusocial systems.

The second step to establish an important role for social immunity in the early evolution of social life is to show that its personal and collective mechanisms are at least partly selected for their collective benefits. To date, however, this important step lacks experimental evidence. One possible reason is that some of the mechanisms of social immunity are known to mediate other key processes in insects, so testing their immune benefits while preventing the expression of their other properties is experimentally difficult. For instance, frass removal can serve to limit parasite establishment in the nesting habitats, but also to eliminate chemical cues used by parasites to detect host presence, or simply to free space from confined areas [56]. Allogrooming not only helps individuals to remove ectoparasites, but also to share chemical compounds with other nest-mates and consequently to improve the accuracy of nest-mate recognition by homogenizing chemical signatures among group members [108]. Finally, increasing genetic diversity prophylactically limits the spread of parasites among group members, but also shapes the level of social conflicts (e.g. between queens and workers over male production [109]) and improves the efficiency of division of labour [110].

Another reason for this lack of empirical evidence is that most of the currently available data come from eusocial insects, which is not ideal to determine whether social immunity is a driver or a by-product of the evolution of group living [23]. Testing this question indeed requires researchers to disentangle the fitness of the donor individual from that of the recipient, which is experimentally difficult (if not impossible) when the vast majority of group members is sterile or never reproduces, as in eusocial insects. To circumvent this difficulty, one option is to study the benefits of collective forms of immunity in queens that found a new colony (e.g. [111]). However, the specificity of the foundation phase might not properly reflect the selection pressures shaping ancestral eusocial colony life. Another option is to study the benefits of collective forms of immunity in non-eusocial species [23], as their group members traditionally express full reproductive capabilities and thus have experimentally quantifiable individual fitness. Compared to eusocial species, the non-eusocial ones can also be used to investigate temporal trade-off between social and personal immunity, as their group members possibly experience solitary and group living phases during their life cycle. For instance, solitary phases alternate with group living phases in the migratory locust, solitary life takes turns with family life in subsocial insects such as burying beetles, and juveniles even have the capability to choose between family or solitary life in the European earwig *Forficula auricularia* [112]. Finally, increasing the number of studies on social immunity in non-eusocial species would permit phylogenetic studies that are balanced across taxonomic groups and social systems, and, thus, to properly trace the early evolution of collective defences in insects.

In conclusion, using an up-to-date appraisal of the multiple forms of collective immunity reported in group living insects, this review highlights the fact that collective immunity is not limited to eusocial species (see also [23]). The distribution of these mechanisms across the continuum from solitary to eusocial insects suggests that social immunity is a tenet of social evolution, even if some collective mechanisms could be by-products of derived forms of group living. This review also emphasizes that the vast majority of studies on social immunity have focused on eusocial taxa, which calls for future empirical and theoretical research investigating its occurrence across a broader spectrum of group living species. Such additional studies would be particularly interesting, as they would allow conducting phylogenetic studies to reconstruct the evolutionary pathways and timing of collective defences and would more generally offer a novel framework to better

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understand the role of parasite pressures on the emergence, transformation and/or disruption of social life.

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Original Article

Maternal care provides antifungal protection to eggs in the European earwig

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Many insects raise their offspring on organic substrates or in the soil where microorganisms are abundant. Microbes may pose a serious threat to offspring development and survival by either decomposing food resources or directly infecting the offspring. Selection to cope with these effects may favor social defenses, for example, through forms of parental care that can limit or eliminate these threats to offspring fitness. In this study, we experimentally tested if maternal egg attendance in the European earwig *Forficula auricularia* has a function as a social defense against mold infection of eggs by manipulating exposure of eggs to mold spores and the presence of the mother in a fully factorial design. Furthermore, we investigated the potential roles of egg grooming behavior and maternal transfer of chemicals as underlying mechanisms. As predicted, the beneficial effect of egg attendance on hatching success was significantly enhanced when eggs were exposed to the mold. Females significantly increased their egg grooming duration in response to mold exposure of her eggs, and the quantity of chemicals (identified as hydrocarbons) was maintained among attended eggs but decreased substantially among unattended eggs. Maternal transfer of chemicals was confirmed in extractions of glass beads that were mingled into attended or unattended clutches. This study shows that maternal egg attendance in the European earwig has a social defense function protecting offspring against mold infection. The maternal egg grooming behavior seems to be key for this effect, probably through both the mechanical removal of spores and the continued application of chemical substances on the egg surface.

Key words: **antimicrobial defense, Dermaptera, egg attendance, egg grooming, insect, *Mucor*, social defence, social evolution.**

INTRODUCTION

Across animal species and taxa, a broad variety of mechanisms are known to help individuals limit their risks of infection by parasites and microbial pathogens. These mechanisms include parasite avoidance, self-grooming, or specific and nonspecific immunological responses (Schmid-Hempel 2003; Schmid-Hempel and Ebert 2003; Cremer and Sixt 2009; Cotter and Kilner 2010a). In addition to individual defenses against infection, recent studies demonstrated that socially mediated collective mechanisms also evolved in group-living organisms. Forms of social immunity are expected because the frequent and intimate contacts between individuals in group-living organisms facilitate disease transmission. An added factor specific to kin groups (e.g., eusocial insect colonies) is that the close genetic relatedness between group members may render them susceptible to the same pathogens (Schmid-Hempel 2003; Cremer et al. 2007). A very common behavioral form of social immunity is allogrooming, during which individuals groom other individuals to remove, for example, pathogenic fungi on the surface of other group members or to apply antimicrobial chemical substances (Rosengaus et al. 1999; Ugelvig et al. 2010; Reber et al. 2011; Tragust et al. 2013). Allogrooming can also mediate pathogen exposure of the grooming individuals which can enhance their own resistance on a later exposure (Ugelvig and Cremer 2007; Konrad et al. 2012).

Whereas mechanisms of social immunity received growing attention in eusocial insects such as ants, bees, or termites (Traniello et al. 2002; Cremer et al. 2007; Ugelvig and Cremer 2007; Reber et al. 2011), there is comparably little research on social defense mechanisms in non-eusocial systems, such as families where parents care for their own offspring. In families, parasites pose a potential threat to offspring fitness, and selection may favor forms of parental care that include mechanisms of social defenses against detrimental effects of parasites on offspring. For example, in birds, females deposit hormones or antibodies to the eggs protecting their offspring from infections during early development (e.g., Grindstaff et al. 2003). Or in some insect species with parental care, parents transfer antimicrobial substances to their offspring's food sources to prevent this food from degradation and, hence, to

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reduce offspring competition with microbes (Trumbo 2012). For example, in the burying beetle *Nicrophorus vespilloides*, parents protect the vertebrate carcass on which they breed from bacterial degradation by using secretions from their anal glands (Rozen et al. 2008; Cotter and Kilner 2010b; Arce et al. 2012). In the beewolf digger wasps *Philanthus triangulum*, females embalm the food provisioned to their offspring (paralyzed honeybees) with hydrocarbons that prevent water condensation and thereby inhibit mold growth (Herzner and Strohm 2007). Furthermore, females provide secretions to the brood cell cover containing symbiotic bacteria from their antennal glands. In many cases, these bacteria are then taken up by the larvae and later incorporated into their cocoon as a protection against fungal or bacterial infections (Kroiss et al. 2010).

A widespread form of parental care in insects is egg attendance (Trumbo 2012; Wong and Kölliker 2012; see Royle et al. 2012). Whereas parental egg attendance is generally known to protect eggs against inter- or intraspecific predation (Cocroft 1999; Zink 2003; Miller and Zink 2012; Trumbo 2012; Wong and Kölliker 2012), little is known about its protective function against egg infection by fungi and bacteria (Costa 2006; Trumbo 2012). Fungi and bacteria may be key ecological agents of selection for parental egg attendance in insects, as many species raise their clutch of eggs on organic substrates or in burrows/tunnels in the soil where they are continuously in contact with bacteria, fungi, and mold (Costa 2006; Cremer and Sixt 2009; Reber and Chapuisat 2012; Trumbo 2012). These microbes all pose potentially serious threats not only indirectly by decomposing food sources (see above) but also directly by infecting the eggs and/or impacting embryonic development and survival.

An antifungal/parasitic function of egg attendance (in particular egg grooming) by parents or workers has been suggested in several insect species (e.g., Costa 2006; Trumbo 2012), but only few experiments have been carried out to address this question directly using an experimental approach. In the ant *Formica selysi*, workers increase their egg grooming behavior in response to exposure to pathogens (Reber et al. 2011), and earwig (Dermaptera) females exhibit a characteristic egg grooming behavior when they tend their clutch (Costa 2006). In the ring-legged earwig *Euborellia annulipes* (Klostermeyer 1942) and the maritime earwig *Anisolabis maritima* (Miller and Zink 2012), unattended eggs were shown to be more often infected by mold than attended eggs. However, because mold exposure was not experimentally manipulated, these latter results did not allow disentangling whether egg attendance was causal in reducing mold infection, or whether it enhanced egg survival through another function, leading to higher egg mortality in unattended clutches and enhanced opportunistic mold growth on already dead eggs. Buxton and Madge (1974) carried out an experiment to test if maternal egg grooming in the European earwig *Forficula auricularia* is to mechanically remove mold spores. Their mechanical treatment of eggs with a paintbrush reduced mold infection, but it is uncertain if the brush treatment is a meaningful imitation of the mechanical effect of maternal egg grooming. Also, it remains to be tested whether females use other forms of protection, for example, by applying specific chemicals on the egg surface (Herzner and Strohm 2007; Matsuura et al. 2007; Tragust et al. 2013) to limit the risks of microbial infections.

In this study, we used a combination of behavioral experiments and chemical extraction and quantification methods to test the antimicrobial function of maternal care in the European earwig (*F. auricularia*) and to investigate potential underlying behavioral and chemical mechanisms. The European earwig is ideal to address this question because 1) females attend their clutches in burrows in the soil with the corresponding exposure of eggs to soil fungi and other microorganisms, 2) females show a characteristic egg-grooming behavior, 3) previous studies suggest maternal care is required for successful egg hatching (see above), and 4) many of the life history traits of this species have been well studied (Lamb and Wellington 1975; Lamb 1976; Costa 2006; Kölliker 2007; Meunier and Kölliker 2012; Meunier et al. 2012). By conducting a full-factorial experiment in which we manipulated the presence/absence of the attending mother, as well as the exposure of eggs to mold spores, we tested the predictions that 1) the beneficial effect of maternal egg attendance on offspring fitness (in terms of hatching success and hatchling body weight) is enhanced when the eggs are exposed to mold spores, 2) females respond to spore exposure of their eggs by increasing the duration of maternal egg grooming, and 3) mothers transfer chemical compounds to the eggs that may directly or indirectly protect the eggs against mold growth.

MATERIALS AND METHODS

Origin of the tested individuals

The *F. auricularia* adults used in our experiments were from the fifth generation of a large population reared in the laboratory and originating from a population that was collected in the field in Dolcedo (Italy) in May 2009 (Meunier et al. 2012). The laboratory population was maintained, each generation, by breeding newly produced adults distributed over plastic containers (37 × 22 × 25 cm), in which groups of 24 virgin females were allowed to mate with 24 unrelated males. During the adult stages, these populations were maintained under a 14:10 h light:dark cycle, at constant 20 °C and 70% relative humidity, and were fed twice a week with the standard food used for the laboratory populations (consisting of Agar-Agar, carrots, bird and dry cat food, wheat germ, cooked egg yolk, ascorbic and sorbic acid) (Meunier et al. 2012). When the first female was observed laying eggs in its original plastic container, all females were isolated in individual Petri dishes containing humid sand as a substrate. Water was added as necessary to keep the sand humid. The dishes were maintained in complete darkness, first under 10 °C for 2 weeks for initiation of oviposition, and then at 15 °C and 70% humidity afterward for egg laying and until hatching (Meunier et al. 2012). Females had no access to food from egg laying to hatching (Kölliker 2007; Meunier et al. 2012).

Origin and identification of the tested mold

The mold used to manipulate the presence of spores on eggs (see below) was initially collected in the containers where we held the earwigs. The mold mainly grew on earwig food or frass. We then cultivated the mold on our standard earwig food under 10:14 h light:dark cycle, 20/15 °C, and 70% relative humidity. The mold spores involved in this experiment originated from 5-day-old cultures, and they were transferred for exposure to filter papers by harvesting the spores of the mold sweeping a Pasteur pipette first over the mold and then rubbing the spores from the pipette onto the filter papers. After an initial morphological identification of 14 sporulated isolates, the reliability of this identification was confirmed using molecular methods (see Supplementary Materials). It is well known that humidity affects mold growth (e.g., Mari et al. 2000), which is why we carried out a preliminary study where the effects of mold exposure and maternal attendance were tested

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under 2 different levels of humidity (i.e., very high and relatively low amounts of water added to the sand substrate). Because humidity had no significant effect on how mold exposure affected hatching success (n = 101; P = 0.609; Boos S, Kölliker M, unpublished data), we carried out the main study under the regular laboratory conditions.

Experiment 1: effects of maternal egg attendance with and without mold exposure

The effects of maternal egg attendance and egg mold exposure on hatching success and offspring quality (hatchling body weight) was tested in 50 clutches, each split in 2 halves, using a split-clutch design (Figure 1). Two factors were manipulated: 1) the presence/absence of the attending mother (within [split]-clutch treatment) and 2) the exposure to mold spores/sham treatment of the eggs (between-clutch treatment). The clutches were randomly assigned to the spore exposure treatment or control on the second day after oviposition, resulting in 25 clutches where the eggs were later exposed to mold spores and 25 clutches where the eggs were sham treated. The mean (± standard deviation [SD]) clutch size was 66.84 (± 7.16) eggs. Each clutch was then split into 2 sets of approximately 31 eggs each (on average 31.80 ± 2.26 [mean ± SD] eggs). The eggs of each egg set were then transferred into a new Petri dish with humid sand as substrate as before, and either with mother (maternal presence treatment) or without mother (maternal absence treatment). The experimental groups were then maintained under standardized laboratory conditions (see above).

The mold spore exposure treatments took place 8 days after oviposition. The split clutches were each separately isolated for 60 min for exposure treatment during which the eggs of each set were moved gently 3 times over a filter paper (Whatman, Sigma-Aldrich, Buchs, Switzerland; diameter 4.25 cm) with a spatula. The filter papers were either untreated (control) or mold spores were previously spread over the filter paper (spore exposure treatment). To confirm successful transfer of spores onto the eggs and to ensure that the treatment did not cause damage to the eggs, we carefully checked pictures of each set of eggs taken with a Leica MZ 12.5 stereomicroscope after treatment (magnitude ×1.0). Spores/sporangia were easily visible as black speckles on the white surface of the eggs (Boos S, personal observations). After the exposure treatment, each set of eggs was transferred to a new Petri dish (diameter: 6 cm) either with or without their mother (according to the maternal presence treatment).

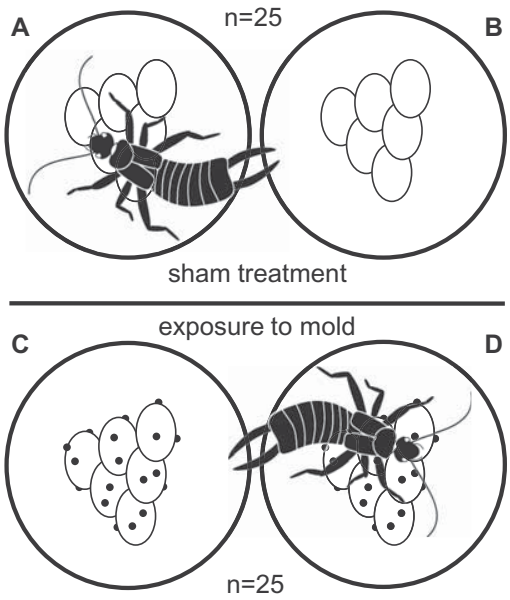

Figure 1
Graphical illustration of the experimental design. Each clutch was split into 2 sets of approximately 31 eggs each (A and B or C and D), which were then assigned 1 of 4 treatments: (A) maternal attendance and no application of spores, (B) no maternal attendance and no application of spores, (C) no maternal attendance and application of spores, and (D) maternal attendance and application of spores. Black dots in (C) and (D) symbolize mold spores/sporangia. n refers to the number of replicates per treatment.

Directly after this exposure treatment, we recorded female behavior under infrared light using a Sony HDR SR8E Video camera (mode: SD/LP) for 75 min. From the recordings, the total time spent egg grooming was quantified by playing the movie sequences with the software QuickTime player (Version 10.0) with an 8-fold time lapse. The first 2 min of each video were discarded as habituation phase. The egg grooming measurements were highly repeatable (r = 0.99 using 10 randomly chosen females scored twice; Lessells and Boag 1987).

After filming, the eggs and females (if any, depending on treatment) were returned into their Petri dishes and held under standard laboratory conditions until hatching (generally occurring 2 weeks later). One day after the first egg hatched, the total hatching success and offspring quality per clutch were assessed by counting the number of hatched nymphs and weighing all nymphs to the nearest 0.01 mg using a Mettler Toledo MT5 high precision balance, respectively.

Experiment 2: effect of maternal presence on chemical compounds on egg surface

This series of 2 experiments aimed at testing whether mothers transfer chemical compounds to their attended eggs. In the first, we investigated the influence of maternal egg attendance on the quantity of chemical compounds present on the surface of eggs using a split-clutch experiment. Similarly to experiment 1, the 2-day-old clutches of 20 females were split into 2 sets of approximately 29 eggs each (on average 29.25 ± 3.30 eggs) and then transferred into a new Petri dish either with their mother (maternal presence treatment) or without mother (maternal absence treatment). Changes in the quantity of chemical compounds present on the respective eggs were then tested by randomly sampling 5 pairs (i.e., sets of eggs with and without their own mother) on day 2, 8, 14, and 20 after setup and then extracting the chemical compounds present on the eggs. The difference in the chemical profile of eggs attended by females and unattended eggs is indicative for maternally transferred chemical substances.

To confirm maternal transfer, we ran an additional experiment in which 10 glass beads (diameter 1.25–1.65 mm, Roth, Arlesheim, Switzerland) were mixed with the eggs directly after oviposition in each of 5 clutches (i.e., in 10 split-clutches with and without mother). The females in all cases accepted the glass beads and showed similar tending behavior as toward eggs (Boos S, personal observations; Worthington 1926; Butnariu et al. 2013). The chemicals on the surface of the glass beads were extracted 20 days after setup. The presence of chemical compounds on the glass beads in the maternal presence treatment and their absence in the maternal absence treatment would demonstrate the transfer of chemical compounds from the females to their eggs. We conducted the same handling and extraction in the maternal absence treatment to control for potential contaminations from other eggs.

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Chemical extractions of the eggs

Eggs (or glass beads) were extracted for 6 min in 200 µL extraction solution consisting of 1.25 ng/µL of an internal standard (*n*-octadecane, $C_{18}H_{38}$, Sigma-Aldrich, Buchs, Switzerland) in *n*-heptane (C_7H_{16}, Roth, Arlesheim, Switzerland). Hundred microliters of each extract was stored at −28 °C until analysis. Chemical analysis was carried out with an Agilent7890 gas chromatograph coupled to an Agilent5975C inert XC MSD mass spectrometer. Two-microliter aliquots of each extraction sample were injected using splitless mode on a DB5 column (HP-5ms: length: 30 m, inner diameter: 0.25 mm, film thickness: 0.25 µm, Agilent Technologies, Basel, Switzerland) with a SSL injector temperature held constant at 250 °C and a helium flow rate of 1 mL/s. GC oven temperature started at 70 °C and was held for 2 min. The temperature was then ramped at different rates over the course of the run: It was initially raised at 15 °C/min to 232 °C where it was held for 11 min. In a next step, temperature increased at 5 °C/min to 263 °C and then at 15 °C/min to 300 °C where the temperature was finally held for 7 min.

We focused on a qualitative identification of compounds to identify the chemical class of the found compounds and the most abundant peaks by comparing the mass spectra of the peaks with the mass spectral library NIST2008.

Statistical analysis

The statistical analyses were conducted using R (Version 2.15.2). The influence of the maternal presence and spore exposure treatments on hatching success (calculated through the *cbind(x,y)* function in *R*, in which *x* was the number of nymphs at hatching and *y* the number of eggs that did not hatch [total eggs − nymphs at hatching]) and the mean weight of nymphs were analyzed using 2 generalized linear mixed models (GLMMs), with binomial error distribution (linked with a logit transformation) and with Gaussian error distribution (linked with identity), respectively. In these models, maternal presence, spore exposure, and their interactions were entered as fixed factors, and the clutch identity (ID) as a random factor to accommodate the statistical dependencies arising from the split-clutch design (using glmmPQL; package car Version 2.0-16). Two clutches were excluded from the analysis of hatching success because the mothers ate the eggs. Egg grooming behavior was analyzed using a linear model with the exposure treatment as fixed factor. The change of the chemical compounds on the egg surface over time and the influence of maternal presence on this pattern were also analyzed using a GLMM (with Gaussian error distribution and an identity link), in which the maternal presence treatment was entered as a fixed factor, the day of extraction as covariate, and the clutch ID as a random factor. Glass beads were analyzed with a linear model with the maternal presence treatment as a fixed factor. The quantities of the chemical compounds on the egg surface and glass beads were cubic root transformed for analyses.

RESULTS

Experiment 1: effects of maternal egg attendance with and without mold exposure

As predicted, if maternal egg attendance has an antifungal function, the beneficial effect of maternal presence on hatching success was significantly enhanced when the eggs were exposed to spores. This is shown by a significant effect of the interaction between the maternal presence and the spore exposure treatment on hatching success (Figure 2A; $\chi^2_1 = 55.34$, $P < 0.001$; main effect maternal presence treatment: $\chi^2_1 = 138.08$, $P < 0.001$; main effect spore exposure treatment: $\chi^2_1 = 59.85$, $P < 0.001$). This interaction arose because spore exposure significantly decreased hatching success in unattended clutches ($\chi^2_1 = 130.21$, $P < 0.001$) but had

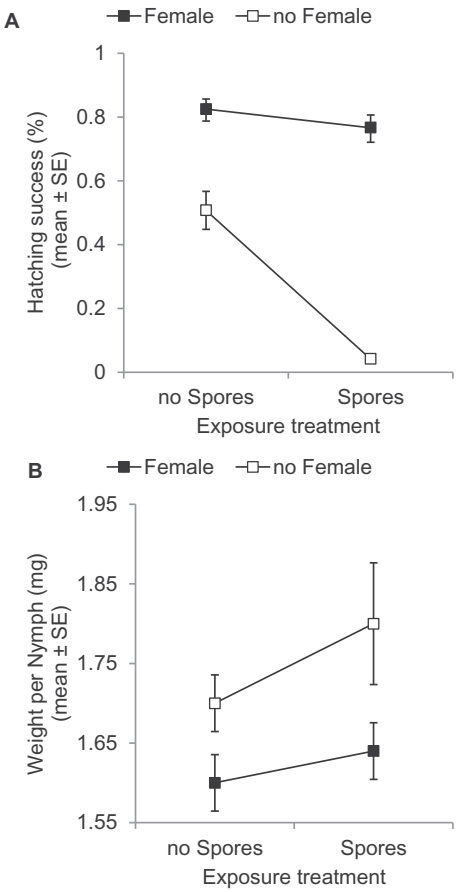

Figure 2
Effects of mold spore exposure of eggs and maternal presence on hatching success (A) and mean hatchling body weight (B). Shown are means and standard errors. The results are in relation to the spore exposure treatment. Filled symbols are for the treatments where the mother was present, open symbols for the treatments where the mother was absent (see variable legend).

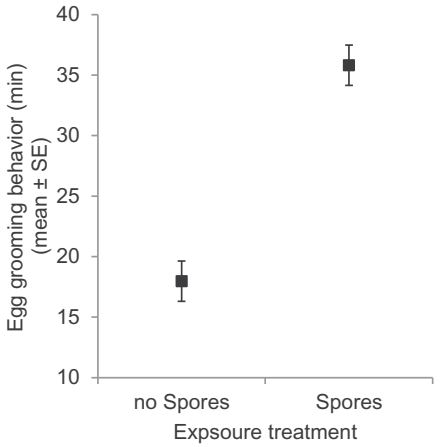

Figure 3
Effect of mold spore exposure on the duration of maternal egg grooming. Quantification of egg grooming was made over the course of a 75-min observation period. Shown are means and standard errors.

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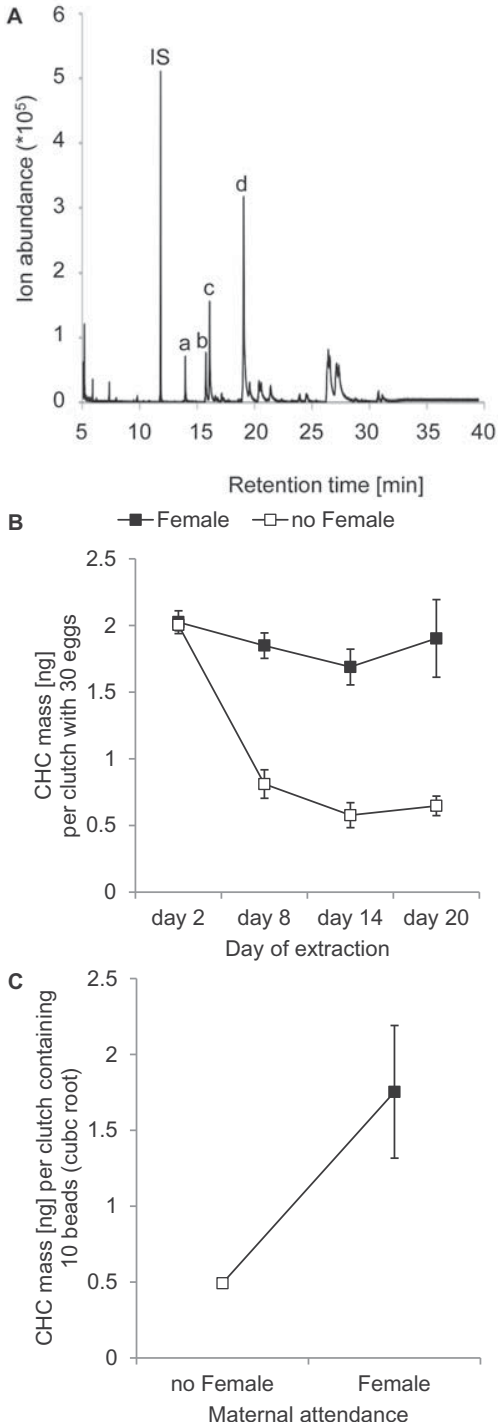

Figure 4
Maternal transfer of chemical compounds to the egg surface. (A) Exemplary chromatogram (total ions) of the extract from an attended clutch. Most of the visible peaks were hydrocarbons. The major peaks were identified as (a) heneicosane ($C_{21}H_{44}$), (b) Z-9-tricosene ($C_{23}H_{46}$), (c) tricosane ($C_{23}H_{48}$), and (d) Z-12-pentacosene ($C_{23}H_{50}$). IS indicates the internal standard *n*-octadecane ($C_{18}H_{38}$). (B) Change of total hydrocarbon quantities over time in clutches attended by their mother (filled symbols), and in unattended clutches (open symbols). (C) Total hydrocarbon quantities on glass beads mingled into maternally attended clutches and unattended clutches. In (B) and (C), means and standard errors are shown.

no significant effect on hatching success in clutches attended by a female ($\chi^2_1 = 1.51$, $P = 0.22$).

Nymph weight at hatching was significantly lower in clutches attended by females than in unattended clutches (Figure 2B; $\chi^2_1 = 4.34$, $P = 0.037$) but was not significantly influenced by spore exposure ($\chi^2_1 = 1.63$, $P = 0.202$) or by an interaction between the maternal presence and the spore exposure treatment ($\chi^2_1 = 0.45$, $P = 0.504$). There was no significant association between nymph weight and clutch size ($\chi^2_1 = 1.16$, $P = 0.281$).

As predicted, if females respond to spores on their eggs, tending females spent significantly more time egg grooming in the spore exposure treatment than in the unexposed control (Figure 3; *t*-test; $t_{48} = 7.59$, $P < 0.001$).

Experiment 2: effect of maternal presence on chemical compounds on egg surface

The chemical compounds extracted from the egg surface (Figure 4A) were mostly hydrocarbons. If these hydrocarbons were at least partly transferred by the female, we expected their quantity to be maintained in the presence of a tending mother and to decrease over time in the absence of a tending mother. This expected pattern was supported by our data. The summed quantity of hydrocarbons did not change significantly in the maternal presence treatment (Figure 4B; $\chi^2_1 = 0.53$, $P = 0.467$) but decreased strongly and significantly over time in the maternal absence treatment (Figure 4B; $\chi^2_1 = 34.92$, $P < 0.001$). The difference in slopes between treatments was significant (interaction effect: $\chi^2_1 = 20.74$, $P < 0.001$; main effect maternal presence: $\chi^2_1 = 3.50$, $P = 0.061$; main effect time: $\chi^2_1 = 35.18$, $P < 0.001$).

Maternal transfer of the hydrocarbons on the egg surface was confirmed in the glass beads experiment. Extracts from glass beads that were mixed into maternally attended clutches contained significantly higher quantities of hydrocarbons than glass beads mixed into unattended clutches (Figure 4C; *t*-test; $t_8 = 2.88$, $P = 0.021$).

DISCUSSION

Egg attendance is a widespread form of parental care among invertebrates (Tallamy 1984; Costa 2006; Trumbo 2012; Wong et al. 2013) and is known to enhance the fitness of eggs through protection against inter- or intraspecific predation (e.g., Pollard 1984; Tallamy 1984; Machado and Oliveira 2002; Zink 2003; Miller et al. 2011; Miller and Zink 2012). The potential role of maternal egg attendance as a form of social defense against parasites/microbes, although proposed repeatedly, was only rarely tested experimentally. Here, we demonstrated in the European earwig that 1) maternal egg attendance strongly and significantly reduced the detrimental effects of spore exposure of eggs on hatching success, 2) spore exposure of eggs significantly increased the duration of maternal egg grooming, and 3) maternal egg attendance lead to a larger overall amount of chemical compounds (hydrocarbons) on the eggs, apparently through continued maternal transfer. Furthermore, we also found 4) that hatchling body weight was lower in attended than unattended clutches but that this effect was independent of mold exposure.

We showed that the presence of the mother particularly enhanced hatching success under mold exposure, demonstrating an antifungal social defense function of female egg attendance. In fact, exposed eggs without tending mother had very low average hatching success of only 4% (compared with 77% with a tending

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mother; Figure 2A), which shows the detrimental effects of mold on offspring fitness. This result, together with the observation that mold occurs commonly in the soil where organic matter is decomposed (e.g., Mari et al. 2000; Gherbawy et al. 2009), suggests a role for soil fungi as agents of selection contributing to the maintenance of maternal egg attendance in earwigs. Our results do not allow direct inference about the role of soil microbes (i.e., mold) in the evolutionary origin of maternal care. It is conceivable that the susceptible eggshell of Dermapterans is ancestral (in conjunction with the secondary loss of an ovipositor among the Neopteran lineage) and maternal egg attendance evolved as a parental care adaptation to protect susceptible eggs from environmental hazards (Zeh et al. 1989) such as mold. However, the current dependence of the eggs on maternal social defenses may not adequately reflect the susceptibility of eggs when this form of maternal care originated. Given maternal protection, it may have secondarily evolved to become more susceptible to infection (see Trumbo 2012) in which case the current benefits of maternal egg attendance would overestimate the benefits characterizing an ancestral state (Smiseth et al. 2012).

Our results clearly show that mothers enhanced offspring fitness under mold exposure of eggs in terms of hatching success. The results on hatchling body weight, a potential measure of offspring quality because heavier nymphs have a survival advantage in cannibalistic interactions (Dobler and Kölliker 2011), were less straightforward. Nymphs hatching from attended clutches were significantly lighter than those hatching from unattended clutches (an effect not significantly influenced by mold exposure). There are several possible explanations for this result. For example, only the highest quality eggs may have hatched in the absence of a mother resulting in less numerous but heavier individuals. This hypothesis would imply that mothers helped during the hatching process, a potential additional function of egg attendance, which has not yet been examined in earwigs. Alternatively, it is conceivable that early hatched nymphs cannibalized later hatched ones (i.e., sibling cannibalism occurs readily in *F. auricularia*; see Dobler and Kölliker 2010), but in this experiment, no cannibalism was observed during the short time window over which hatching of a clutch occurs (approximately 24 h: Boos S, unpublished data). Finally, the effect may be due to a difference in water balance between unattended and attended eggs in which case variation in fresh weight among hatchlings would not necessarily relate to a measure of quality. We showed that mothers transfer hydrocarbons to the egg surface, and it is well known that hydrocarbons on the cuticle of insects are key for water homeostasis (Blomquist and Bagnères 2010). Thus, the low quantities of hydrocarbons on the unattended eggs (Figure 4B,C) might have facilitated passive water absorption by the embryos from the substrate and surrounding air leading to their heavier fresh weight at hatching (Chauvin et al. 1991).

For our spore exposure experiments, we collected the spores from mold growing on the food and frass of the earwigs in our laboratory population. Morphological and DNA barcoding identification confirmed that this mold was part of the genus *Mucor* (see Supplementary Figure 1). *Mucor* is an ubiquitous microbial genus occurring broadly also in the soil (e.g., Mari et al. 2000; Gherbawy et al. 2009; Reber and Chapuisat 2012), has a saprophytic lifestyle, and gains nutrition from the decomposition of organic matter. Furthermore, the *Mucorales* have also been identified in arthropod habitats such as in nests of the Indian and European paper wasp (*Ropalidia maginata*; Jayaprakash and Ebenezer 2010, *Polistes dominulus*; Madden et al. 2012). Given the broad occurrence in the soil (including reports from soil samples in Italy; Mari et al. 2000) and unspecific/opportunistic nature of how this fungus infects or decomposes organic matter, we expect earwigs to be commonly exposed to this widespread mold under natural conditions and hypothesize that the defense shown by earwig females is probably rather unspecific (see below). Further experiments should however test the specificity of maternal egg-grooming behavior and the maternally transferred chemical compounds with regard to different kinds of soil microbes (Reber and Chapuisat 2012).

In terms of underlying mechanisms of maternal defenses, our results suggest roles for the egg grooming behavior and the application of hydrocarbons to the egg surface. As expected, earwig mothers spent significantly more time grooming their eggs when eggs were exposed to mold spores. Thus, females detected the presence of the fungal spores and flexibly responded by a 2-fold increase in their grooming duration. It is noteworthy that after mold application and the observation of egg grooming behavior, eggs were always free of mold (Boos S, personal observations), suggesting that earwig mothers removed the mold spores mechanically by egg grooming. Maternal egg grooming correspondingly has a function that is analogous to allogrooming in ant and termite colonies (Matsuura et al. 2007; Ugelvig and Cremer 2007; Tragust et al. 2013).

The duration of egg grooming in the absence of fungal spores was still quite substantial (18 min out of 75-min observation). It is conceivable that this grooming may at least partly reflect a response to other microbes that were present on the eggs. Alternatively, egg grooming may have additional functions that explain this baseline level of egg grooming such as the application of hydrocarbons for egg water homeostasis (see above).

Whether females transferred chemical compounds during egg attendance was tested by comparing the chemical profiles of heptane extracts between attended and unattended eggs, and between glass beads mingled into attended and unattended clutches. In both cases, all the identified chemical compounds were hydrocarbons. Their total extracted quantity decreased quickly in unattended clutches, whereas it was maintained in attended clutches. This effect lead to a significantly higher quantity of hydrocarbons on attended eggs within 6 days, an effect increasing in magnitude over the course of time. This is evidence that earwig mothers progressively transfer hydrocarbons to their eggs during egg attendance, maintaining a constant amount on the egg surface, whereas unattended eggs lost a substantial fraction over the course of a few days. It seems likely that the chemicals were provided with the saliva via egg grooming, but we cannot exclude other ways of application such as the spraying of eggs with secretions from their dorsal abdominal glands (Eisner 1960; Eisner et al. 2000; Gasch et al. 2013). Irrespective of the transfer mechanism, the hydrocarbons may contribute to egg protection against mold through the indirect mechanism proposed by Strohm and Linsenmair (2001) whereby the application of hydrocarbons prevents water condensation on the surface resulting in a suboptimal microclimate for mold germination and growth.

Based on our extraction and analytical methods, we did not find chemical compounds with known antibiotic/antifungal properties and we did not test for peptides, lysozymes (Matsuura et al. 2007; Rozen et al. 2008; Cotter and Kilner 2010b), or symbiotic bacteria (Kroiss et al. 2010) that may protect eggs against mold infection. A recent study demonstrated experimentally the antimicrobial activity of the general defensive secretions of *F. auricularia*, arguing that the contained benzoquinones play an important role (Gasch et al. 2013). One could also speculate that some bacteria surrounding the

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earwig eggs and potentially delivered by the mother may produce antimicrobial peptides to protect eggs from fungus infection (analogous to the processes in the paper wasp *Polistes dominulus*; Madden et al. 2012 or the beewolf digger wasps *P. triangulum*; Strohm and Linsenmair 2001). Clearly, a full understanding of the chemical defense in a maternal care context will require further research on transferred compounds and their antimicrobial properties.

To conclude, we demonstrated that maternal egg attendance includes forms of social defenses against the infection and decomposition of eggs by mold in the European earwig *F. auricularia*. Our results overall suggest that egg grooming is a maternal behavior that may generally serve both a mechanical removal of egg spores by the females' mouthparts and the application of chemicals through transferred saliva (Costa 2006). The former may be a mechanism to quickly defend against an immediate threat (i.e., spores already present on the eggs), and the latter possibly a longer term protection to prevent or reduce scope for spores to adhere to or germinate on the eggs. Further research is needed to disentangle the role of mechanical and chemical maternal defenses as well as their specificity. It is expected that maternal (social) defenses as described here in *F. auricularia* should be widespread in arthropods that breed in the soil or on organic material because of the high expected prevalence of egg exposure to soil microbes that decompose organic material (such as eggs) (Costa 2006; Rozen et al. 2008; Arce et al. 2012; Reber and Chapuisat 2012; Trumbo 2012). Thus, the antifungal/antimicrobial function of egg attendance may be key to explain why egg attendance is such a widespread form of parental care in insects/arthropods.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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Age, pathogen exposure, but not maternal care shape offspring immunity in an insect with facultative family life

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Abstract

Background: To optimize their resistance against pathogen infection, individuals are expected to find the right balance between investing into the immune system and other life history traits. In vertebrates, several factors were shown to critically affect the direction of this balance, such as the developmental stage of an individual, its current risk of infection and/or its access to external help such as parental care. However, the independent and/or interactive effects of these factors on immunity remain poorly studied in insects.

Results: Here, we manipulated maternal presence and pathogen exposure in families of the European earwig *Forficula auricularia* to measure whether and how the survival rate and investment into two key immune parameters changed during offspring development. The pathogen was the entomopathogenic fungus *Metarhizium brunneum* and the immune parameters were hemocyte concentration and phenol/pro-phenoloxidase enzyme activity (total-PO). Our results surprisingly showed that maternal presence had no effect on offspring immunity, but reduced offspring survival. Pathogen exposure also lowered the survival of offspring during their early development. The concentration of hemocytes and the total-PO activity increased during development, to be eventually higher in adult females compared to adult males. Finally, pathogen exposure overall increased the concentration of hemocytes—but not the total-PO activity—in adults, while it had no effect on these measures in offspring.

Conclusions: Our results show that, independent of their infection risk and developmental stage, maternal presence does not shape immune defense in young earwigs. This reveals that pathogen pressure is not a universal evolutionary driver of the emergence and maintenance of post-hatching maternal care in insects.

Keywords: Developmental stage, Instar, Family life, *Forficula auricularia*, Insect immunity, *Metarhizium brunneum*, Trade-off

Background

Most living organisms are parasites [1]. By altering the growth, fecundity, and survival of their hosts, they represent a strong selective force that drives the evolution of multiple defense in their hosts [2]. To limit the costs of pathogen infections, hosts typically depend on their immune system [2]. In insects, an important part of this defense relies on the coordinate action of non-specific and constitutive mechanisms, among which textbook examples involve hemocytes and phenoloxidase [3]. Hemocytes are immune cells that circulate in the hemolymph and are involved in recognition and encapsulation of pathogens [4]. Conversely, phenoloxidase mostly mediates the melanization of foreign objects and operates through the activation of the prophenoloxidase cascade, its inactive precursor typically stored in the hemolymph and the hemocytes [5].

Investing into immunity is costly and individuals are thus expected to adjust this investment to their current risk of infection, their general condition and/or their potential access to external help provided by group members [6]. Many vertebrates and invertebrates were shown to prophylactically increase their investment into immunity when the risk of infection is high, e.g. due to the presence of pathogens or of possibly infected individuals

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in their vicinity [7–9]. For example, populations of the small ground finch *Geospiza fuliginosa* that lived on islands with a high parasite prevalence invested more in their immune system compared to birds under low parasite pressure [10]. Conversely, individuals from the same species/population experiencing favorable conditions either during development and/or adult life are also often able to invest more into energetically costly traits, such as immune defenses [11, 12]. In line with this prediction, large and/or well-nourished individuals are typically known to exhibit higher concentrations of immune components in their blood or hemolymph than small and light ones at the population level [12, 13]. Finally, how much an individual invests into its immunity may also depend on the help it has received or will receive from others, i.e. on the expression of social immunity [14, 15]. Social immunity is a well-studied phenomenon in eusocial insects, where it can take multiple forms such as allo-grooming and hygienic behaviors [14, 16], but is also known to play a central role in simple family units via parental care. The effect of parental care on offspring immunity is well documented in vertebrates, with examples showing that post-hatching parental care enhances the immune response of young barn swallows *Hirundo rustica* [17] or that parental deprivation reduces the immunocompetence of juveniles in mice [18] and rats [19]. Comparatively, the effects of post-hatching parental care on offspring immunity are less clear in invertebrates, with only one study showing that parental deprivation reduces the lytic activity of larval exudate—a mediator of social immunity—in the burying beetle *Nicrophorus vespilloides* [20].

Interestingly, the influence of parental care on offspring immunity may depend on the age of the offspring and their risks of pathogen infection. In many vertebrates and invertebrates, immunocompetence increases during development [21–24]. Consequently, the effects of parental care on offspring immunity could be limited to the early stages of development (when parents interact with their juveniles) and then disappear when these juveniles have developed their own immune defenses. On the other hand, parental care facilitates offspring development with effects often reaching into adulthood, so that immune defenses could still be altered long after parents stopped caring for their offspring. Finally, the risk of infection could also determine how much parents invest into the care of their juveniles [25] and thus how much the offspring can invest into their own immune defense. For instance, the presence of pathogens in the environment has been shown to increase the expression of parental care in the frog *Hylophorbus rufescens*, as well as in humans, which in turn results in higher survival rates of pathogen-exposed offspring [26, 27].

In this study, we investigated the simultaneous and interactive effects of early maternal presence, early exposure to pathogens and developmental stage on offspring immunity in the European earwig *Forficula auricularia*. In this insect, females provide multiple forms of care to their juveniles (called nymphs) during 2 weeks following egg hatching, such as food provisioning, allo-grooming and protection against predators [28]. Nevertheless, earwig nymphs are mobile, can forage on their own and are thus typically capable to develop and survive in the absence of a tending mother [28, 29]. Here, we conducted a 2x2 full-factorial experiment in which we manipulated the presence or absence of a mother, as well as the presence or absence of the entomopathogenic fungus *Metarhizium brunneum* in the nest during the two first weeks post egg hatching (i.e. the period of family life). We then measured nymph survival and immune defenses at the 2nd, 3rd and 4th developmental instars, as well as in the adults. Overall, we expect that maternal presence improves the short- and long-term survival of offspring reared in a previously contaminated nest. In terms of immunity, we predict that this positive effect of maternal presence translates in either a maternally-driven increase in the offspring's capability to invest into personal immune defenses (for instance due to the early accumulation of maternally-provided resources) or a maternally-driven decrease of offspring' investment into personal immunity (the immune protection ensured by maternal care could allow nymphs to shift their investment from immunity to other important traits such as growth). Finally, if maternal care has limited or no effect on offspring immunity, we predict offspring immune defenses to increase with age and with early pathogen exposure, but these effects to be independent of early maternal presence.

Methods

Insects rearing

Adult *F. auricularia* earwigs were caught in July-August 2015 in Mainz, Germany (49°58'20.5"N 8°11'42.3"E). Immediately after field sampling, earwigs were distributed among plastic containers (37 × 22 × 25 cm) grounded with humid sand. These adults were then allowed to mate freely for 4 months. Thereafter, all females were removed from their containers to mimic dispersal, a behavior they typically express under natural condition prior to egg laying [30]. The females were isolated in Petri dishes (9 cm diameter) that were furnished with moist sand, maintained under winter conditions (15°C in darkness) and provided with a diet of *ad libitum* standard food (food composition detailed in [31]). Each Petri dish was then checked twice a week for eggs. Food provisioning was stopped when eggs were found, as females typically cease to feed between egg laying and hatching [28]. At egg

hatching, all clutches were transferred to and maintained under summer conditions (18-20°C D:L) until the end of the experiment (conditions detailed in [32]).

Experimental design

A total of 98 clutches were used to measure the effects of early maternal presence and/or early pathogen exposure on two immune parameters on nymphs and young adults. Each clutch was culled to 35 nymphs 1 day after hatching (i.e. 1st instar nymphs) and then transferred to Petri dishes either with (1) their own mother and contaminated sand ($n = 25$), (2) their own mother and non-contaminated sand ($n = 24$), (3) no mother and contaminated sand ($n = 25$) or (4) no mother and non-contaminated sand ($n = 24$). The contaminated and non-contaminated sands were created by preliminary grounding each recipient Petri dish (9 cm diameter) with humid sand and then sprinkling the sand with either 100 μl of a conidiospore solution of *M. brunneum* diluted in 0.05% Tween (10^7 spores/ml) or with 100 μl of a control spore-free solution of 0.05% Tween, respectively. *M. brunneum* is a common entomophagous fungus in the soil, which is known to infect and reduce the survival of a wide range of insects (including earwigs) in nature, but against which the roles of hemocyte concentration and phenoloxidase activity remain largely unclear [33–35]. On day 14 after egg hatching, all tending mothers were removed from their group of nymphs (when applicable) to mimic natural family dispersal [29]. Six days later, each group of nymphs was transferred to a large Petri dish (14 cm diameter) grounded with non-contaminated sand and maintained as such until they reach adulthood. Note that adult males and females produced in each family were separated at emergence to ensure virginity and avoid inbreeding at the time of immune measurements (see below) [36]. All animals were provided with an *ad libitum* amount of standard food changed twice a week (detailed food composition in [31]).

We followed offspring survival during their development by counting all group members either five (2nd, 3rd and 4th developmental instar) or ten (adults) days after the first individual of each clutch molted into the next developmental instar. Note that 1st instar nymphs molt into their 2nd instar approximately 12 days after egg hatching [32, 37]. The days five and ten were chosen to ensure that (almost) all group members reached the new instar (or adulthood) on the day of counting (see details on developmental times in [38]). After counting, we randomly sampled two nymphs per developmental instar (and one adult male and one adult female per group), weighed these individuals to the nearest 0.001 mg using a microscale (model MYA5; PESCALE, Bisingen, Germany), and used them for immune measurements (see below). Note that these animals were subtracted for the calculation of survival rates.

Measurement of the two immune parameters

We measured two key immune parameters in 2nd, 3rd and 4th instar nymphs, as well as in adult males and females: the total-PO activity and the concentration of circulating hemocytes. Total-PO activity was defined as the sum of phenoloxidase (PO) and prophenoloxidase (PPO) activities, therefore reflecting the immunocompetence of an individual in terms of both already activated and not-yet activated phenoloxidase enzymatic cascade. Note that earwig individuals cannot be sexed until they reach adulthood. In each of the two nymphs sampled per instar, between 0.2 to 0.5 μl of hemolymph was first extracted with a glass capillary, while 1μl was extracted in each adult male and female (see above). These extracts were immediately diluted in 11 μl (for nymphs) or 25 μl (for adults) of cold sodium cacodylate/$CaCl_2$ buffer (0.01 M sodium cacodylate, 0.005 M CaCl2; pH 6.5) to measure the two immune parameters.

The concentration of all hemocytes (i.e. independent of their type and thus of their specific immune function) was measured immediately after hemolymph extraction, using 10 μl of diluted hemolymph of the nymph and 10 μl of the diluted hemolymph of each male and female. This counting was done using a Neubauer Improved Haemocytometer and a microscope (magnification x 400), as described in [39].

Total-PO activity was spectrophotometrically measured using a standard protocol described in [39]. Specifically, the diluted hemolymph of one nymph (volume = 1.5 μl) and 16 μl remaining of the diluted hemolymph of each male and female were frozen at -30°C to optimize the measurement of total-PO activity. Each sample of frozen hemolymph was then thawed on ice and centrifuged for 5 min at 4°C (4000 × g). Five μl of the resulting supernatant was then added to a microplate well containing 20 μl of PBS and 140 μl of chymotrypsin solution (Sigma C-7762, 0.07 mg/ml of distilled water). A volume of 20 μl of L-dopa solution (Sigma D-9628; 4 mg/ml of distilled water) was then added to each well. The reaction was allowed to proceed for 2h 47min at 30°C in a microplate reader (Thermo scientific Multiskan™ FC Microplate Photometer). Enzyme activity was defined as the slope of the reaction curve during the linear phase of the reaction (Vmax value: change in absorbance units/min) and measured using the R-based program PO-CALC [40]. All immune measurements were done blind regarding the early presence of the mother and the early exposure to pathogens.

Because the volume of extracted hemolymph and the resulting concentration of hemocytes slightly change between individuals, we standardized the concentration of

hemocytes and total-PO activity (immune parameters) per microliter of hemolymph using the following formula: I x [(Vh + Vb)/Vh]/Vm, in which I is the measured immune parameter, Vh is the volume of extracted hemolymph, Vb is the volume of buffer added (i.e. 11 μl for nymphs or 25 μl for adults) and Vm is the volume applied either to the Haemocymeter for hemocyte count (i.e. 10μl) or on the spectrophotometer plate for total-PO measurement (i.e. 5μl).

Statistical analyses

All statistical analyses were conducted using the software R v3.1.2 loaded with the packages *car, lme4, MASS* and *lsmeans*. The survival rate in between each developmental stage (defined here as "age") of offspring (entered using the *cbind* function) was tested using a generalized linear mixed-effects model (GLMM, with binomial error distribution). In this model, the age (second, third and fourth nymphal instars, and adults), early pathogen exposure (presence/absence) and early maternal presence (presence/absence) were entered as explanatory categorical factors, while the clutch identification (ID) was entered as a random factor to control for the fact that each clutch was used for each age. Because we interested in the survival rate of nymphs until their reach adulthood, adult males and females were pooled as "adults" in this model.

Immune parameters were then analyzed separately for nymphs (for which the sex was unknown) and adults (for which the sex was known). For each nymph and adult data set, hemocyte concentration and total-PO activity were analyzed using two LMMs, in which either the age of the nymphs (second, third and fourth nymphal instars) or the gender of the adults (male or female), early pathogen exposure, early maternal presence and the weight of the measured individual were entered as explanatory factors, whereas the ID was used as a random effect. In nymphs, the weight of each class of age was scaled and centered to correct for the inherent difference in weight between each instar. All models first included all interactions between the explanatory factors and were then simplified stepwise by removing the non-significant interaction terms (all P-values > 0.08). Note that some non-significant interactions are presented here to allow direct comparisons between models, but their removal did not qualitatively change the results.

Results

The presence of a tending mother overall reduced the proportion of offspring that successfully reached adulthood, independent of pathogen exposure and age (Table 1; Fig. 1a). By contrast, offspring survival depended on an interaction between pathogen exposure and age (Table 1; Fig. 1b): the pathogenic fungus *M. brunneum* reduced the survival rate of nymphs to the 2nd and 3rd instar, but did not affect their survival rate between the 3rd and 4th instars and was finally associated with an increased survival rate between the 4th instar and adulthood (Fig. 1b).

Table 1 Effects of age, maternal presence and pathogen exposure on the survival rate of offspring

	Survival	
	Chisq	*p*-value
Age	86.39	**<0.0001**
Maternal presence	4.00	**0.045**
Pathogen exposure	6.21	**0.013**
Age: Pathogen exposure	14.87	**0.002**

Significant *p-values* are in bold. Note that non-significant interactions are not reported in this table

Overall, there were contrasting effects of pathogen exposure, maternal presence, body weight, offspring developmental stage and adult gender on hemocyte concentration and total-PO activity in offspring. Specifically, early pathogen exposure increased hemocyte concentration, but not total-PO activity in adults (Table 2; Figs. 2a and 3a), whereas it did not affect these two immune parameters in nymphs (Table 3; Figs. 2b and 3b). Early maternal presence also had no effect on the concentration of hemocytes and on the total-PO activity in both nymphs and adults (Tables 2 and 3; Figs. 2c, d and 3c and d). By contrast, the association between body weight and hemocyte concentration was positive in nymphs (Table 3; Fig. 4a; $\rho = -0.27$; C.I. 95% = [−0.38;−0.16]), but negative in adults (Table 2; Fig. 4b; $\rho = 0.27$; C.I. 95% = [0.11; 0.41]). There was, however, no association between body weight and total-PO activity in nymphs and adults (Tables 2 and 3). Finally, the concentration of hemocytes and the total-PO activity increased between each nymphal instar (Table 3; Figs. 2f and 3f) and were higher in adult females compared to adult males (Table 2; Figs. 2e and 3e).

Discussion

This study aimed at elucidating the effects of early maternal presence and early exposure to pathogens on the immunity of growing offspring in the European earwig *F. auricularia*. Our results show that the presence of the mother during the first 2 weeks of life has no effect on the immunity of her offspring at both nymphal and adult stages. However, maternal presence generally reduced the survival of their offspring, a result in line with a previous study [38] and suggesting that the benefits of post-hatching care in *F. auricularia* could generally take a form that is excluded from the current experimental setup (e.g. predator defense, thermal resistance). Conversely, we found that early pathogen exposure generally

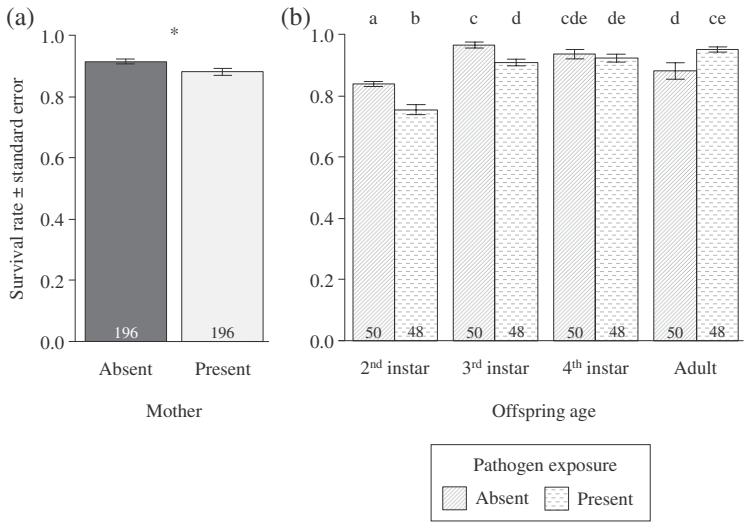

Fig. 1 Effects of maternal presence, offspring age and pathogen exposure on offspring survival rate. The fitted values are given in function of (**a**) maternal presence and of (**b**) the interaction between pathogen exposure and offspring age in between each instar. Sample sizes are provided at the bottom of each bar. Different letters indicate statistically significant differences ($p < 0.05$). * $p < 0.01$

increased the concentration of hemocytes—but not the total-PO activity—in adult offspring and had no effect on nymph's immunity. Both hemocyte concentration and total-PO activities increased with offspring development, and these two immune parameters were higher in adult females compared to adult males. Finally, pathogen exposure early in life caused low survival during the early developmental stages, while it increases adult survival.

Somewhat surprisingly, we found that maternal care has no long-lasting effects on offspring immunity. This result contrasts with other studies investigating short- or long- term effects of parental care/parental deprivation on offspring immunity in vertebrates [17–19, 41]. For instance, nestling immunocompetence increased with parental care 24 h after an immune challenge in barn swallows *Hirundo rustica* [17], and a long-term decrease in immunity has been shown in adult mice early-deprived of their mother [18]. The limited effects of maternal presence on offspring immunity reported here therefore reveal that maternal presence does not necessarily shape the immunity of offspring in insects, and more generally that pathogens are not a universal selective pressure promoting maintenance of post-hatching maternal care in nature. Understanding whether this independence between parental care and offspring immunity is unique to earwig biology [38, 42, 43] (e.g. juveniles are less dependent on maternal care in earwigs compared to most vertebrate and invertebrate species) or on the immune system of invertebrates in general [44] will require further studies exploring the expression and nature of this link across a larger set of species.

Table 2 Effects of gender, maternal presence, pathogen exposure and weight on immune parameters in adults

	Hemocyte concentration		Total-PO activity	
	Chisq	*p*-value	Chisq	*p*-value
Gender	3.97	**0.046**	42.12	**<0.0001**
Maternal presence	0.36	0.547	1.18	0.278
Pathogen exposure	4.99	**0.025**	2.25	0.133
Weight	7.38	**0.006**	0.02	0.900

Significant *p-values* are in bold. Note that non-significant interactions are not reported in this table

Early exposure to *M. brunneum* did not unmask the effect of maternal care on offspring immunity, which contrasts with a previous result demonstrating that maternal presence improves the survival of eggs exposed to fungal spores in this species [25]. However, we found an age-specific effect of pathogen exposure on offspring survival, which reflected a reduced survival rate between hatching and the 3rd instar, an absence of effect between 3rd and 4th instar and a higher survival rate between 4th instar and adulthood. This contrasting age-specific effect of pathogen exposure might reflect three non-mutually exclusive processes. First, juveniles could exhibit a weak immune activity (as reported in many vertebrate and invertebrate species, see for instance in reptiles [45] and in the honey bee [46]), making them less likely to survive pathogen exposure compared to older nymphs [3]. In line with this scenario, we found that the levels of total-PO and hemocyte concentrations increased with the developmental stage of the nymphs. Second, due to our experimental design the 2nd and 3rd

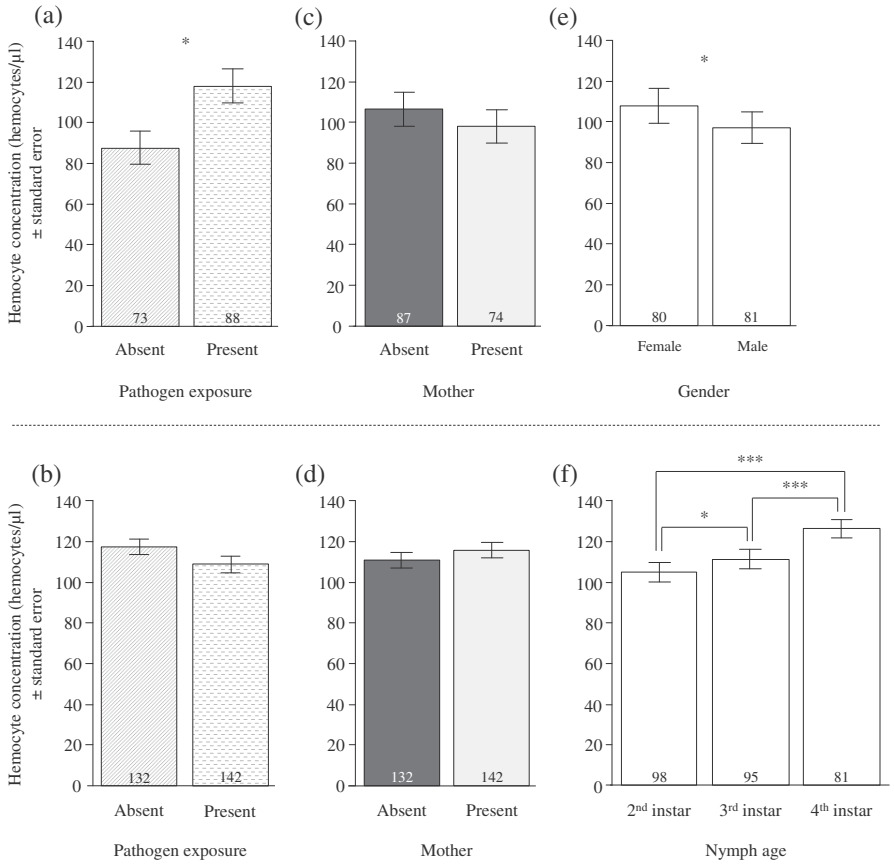

Fig. 2 Effects of pathogen exposure, maternal presence and adult gender or nymphal age on hemocyte concentration. The fitted values are given in function of (**a**, **b**) pathogen exposure, (**c**, **d**) maternal presence and (**e**, **f**) gender/nymph age in adults and nymphs, respectively. White bars represent age/gender, grey hatched/horizontal dotted bars represent the absence/presence of pathogen, and dark/light grey bars represent the absence/presence of mother. Sample sizes are provided at the bottom of each bar. *** $p < 0.0001$

developmental instars were chronologically the first instars emerging after pathogen exposure, which could have resulted in a higher proportion of live spores in the environment of young compared to old offspring and thus in a decreased risk of a novel infection in later instars. Finally, the survival of adults could reflect the early elimination of the weakest individuals in pathogen compared to control treatments, which entailed the production of better quality adults in the former case. To disentangle these three processes, further studies should thus investigate whether offspring exposed to a pathogen either at the beginning of each instar or only at their 1st instar exhibit different or similar survival rates and levels of immunity.

Besides the general increase of offspring's immunity over developmental stages, we found that the association between hemocyte concentration and individual weight was negative in nymphs, but positive in adults. Immunity is generally sustained by either increasing the acquisition of food resources or by reducing energy allocation to other physiological processes such as growth and reproduction [2, 47] (see for examples [17, 47–49]). However, variation in the amount of resources available to an individual is known to possibly mask investment trade-offs between mutually exclusive functions and even to produce positive associations between these functions at a population level [42, 50, 51]. The apparent discrepancy between the presence of a trade-off in nymphs and of a positive association in adults therefore suggests that investing into immunity is generally costly in earwigs, but that this cost is masked in adults—possibly due to a higher variation in resource acquisition between adults compared to between nymphs. Investigating variation in foraging strategies and food intake of nymph and adult earwigs, and thus their role in immune investment will be done in the future. This notwithstanding, our results also reveal that maternal presence does not limit the costs of immune investment, further stressing the limited effects of maternal care on offspring immunity.

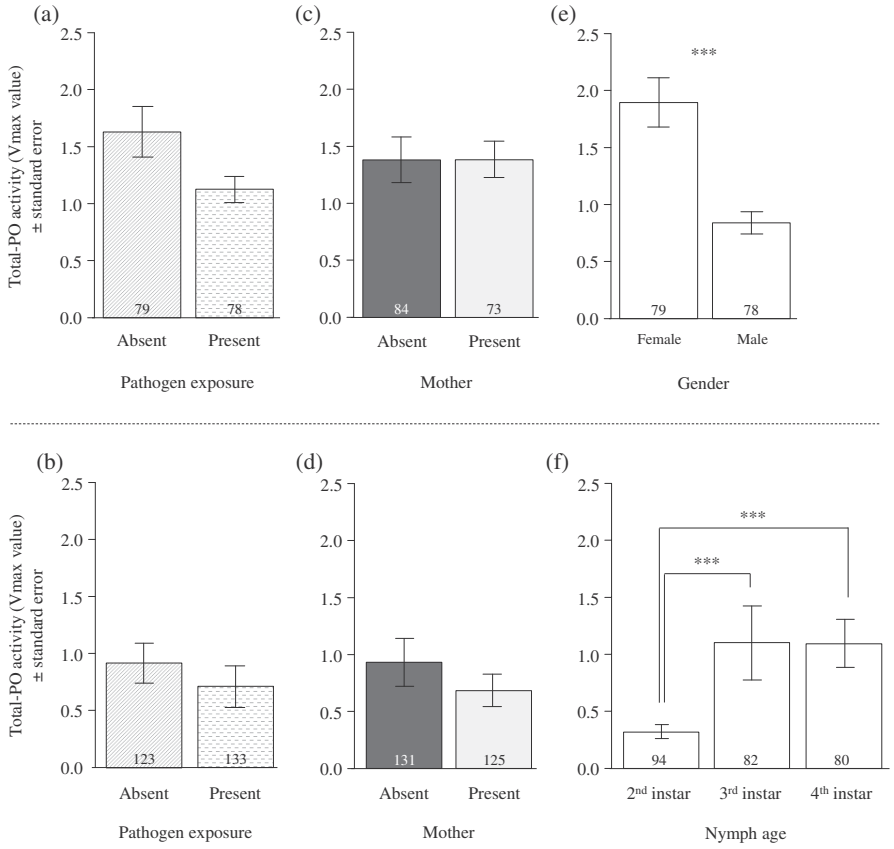

Fig. 3 Effects of pathogen exposure, maternal presence and adult gender or nymphal age on total-PO activity. The fitted values are given in function of (**a**, **b**) pathogen exposure, (**c**, **d**) maternal presence and (**e**, **f**) gender/nymph age in adults and nymphs, respectively. Sample sizes are provided at the bottom of each bar. *** $p < 0.0001$

Independent of body weight, our results finally showed that adult females had more hemocytes and a higher total-PO activity than adult males. This sex-specific investment into immune components is in line with results found across many insect species, such as butterflies [52], crickets [53], dragonflies [54], flies [55] and scorpionflies [56]. In earwigs, males are known to survive only for a single reproductive period (i.e. a few months). By contrast, females live up to 1.5 years, during which they provide care to their eggs for several months, provide care to the resulting nymphs for several weeks and then often produce a second clutch which they care for during several additional weeks [32, 37]. Compared to males, females fitness is therefore tightly associated with their capability to survive over several seasons and thus to fight against longer and/or more frequent attacks by pathogens, overall likely explaining their higher investment into immune defense (see also [33]).

Table 3 Effects of age, maternal presence, pathogen exposure and weight on immune parameters in nymphs

	Hemocyte concentration		Total-PO activity	
	Chisq	*p*-value	Chisq	*p*-value
Age	12.65	**0.001**	74.47	**<0.0001**
Maternal presence	0.41	0.520	0.12	0.732
Pathogen exposure	0.17	0.681	0.61	0.436
Weight	18.71	**<0.0001**	0.13	0.721

Significant *p-values* are in bold. Note that non-significant interactions are not reported in this table

Conclusions

Our results overall reveal that age, gender and parasite exposure shape the immune system of the European earwig *F. auricularia*, while the presence of a caring mother did not. Personal immunity and social immunity in the form of maternal care are nevertheless not the only protection against pathogens that can operate within family units [16]. For instance, larvae can participate in social immunity and thus provide immune benefits to their siblings by sanitizing the nest with anal exudates, a phenomenon reported in the burying beetle *N. vespilloides* [57] and importantly, in the European earwig *F. auricularia* [58]. Our findings therefore reveal that for

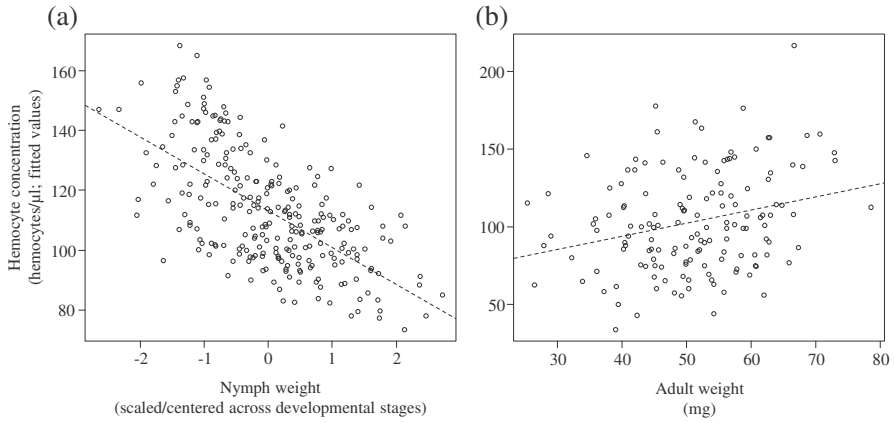

Fig. 4 Correlation between hemocyte concentration and the weight in (**a**) nymphs and (**b**) adults. The hemocyte concentration are fitted values obtained from the LMMs. In nymphs, the weight of each class of age was scaled and centered to correct for the inherent difference in weight between each instar. Each dot represents an individual and the dash line represents correlation between hemocyte concentration and weight

nymphs, the net benefits of family interactions in terms of protection against pathogen infection are unlikely to come from the mothers, but could instead result from the presence and/or interactions with their siblings. Hence, our findings overall call for further studies investigating the role of sibling behaviors, together with age, gender and parasite exposure, in the emergence and maintenance of family life in nature.

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Authors' contributions
FV, MK, SF and JM conceived the experiments; FV and MK performed the experiment; FV and JM performed the statistical analysis; SF provided lab facilities; FV and JM wrote the first draft of the manuscript. All authors helped to improve the final manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable

Ethics approval and consent to participate
Not applicable

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PO-CALC: A novel tool to correct common inconsistencies in the measurement of phenoloxidase activity

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ABSTRACT

A broad range of physiological and evolutionarily studies requires standard and robust methods to assess the strength and activity of an individual's immune defense. In insects, this goal is generally reached by spectrophotometrically measuring (pro-) phenoloxidase activity, an enzymatic and non-specific process activated after wounding and parasite infections. However, the literature surprisingly lacks a standard method to calculate these values from spectrophotometer data and thus to be able to compare results across studies. In this study, we demonstrated that nine methods commonly used to extract phenoloxidase activities (1) provide inconsistent results when tested on the same data sets, at least partly due to their specific sensitivity to the noise regularly present in enzymatic reaction curves. To circumvent this issue, we then (2) developed a novel, free and simple R-based program called *PO-CALC* and (3) demonstrated the robustness of its calculations for the different types of noises. Overall, we show that *PO-CALC* corrects overlooked though important inconsistencies in the measurement of phenoloxidase activities, and claim that its broad use would increase the significance and general validity of studies on invertebrate immunity.

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1. Introduction

Measuring insect's immunocompetence is a central tenet of a broad range of physiological and evolutionary studies. For instance, this measurement is used to study environment and nutrition dependent immunocompetence (Catalán et al., 2012; Bauerfeind and Fischer, 2014), ontogenic maturation and development of the immune system (Piñera et al., 2013), but also host-pathogen coevolution (Eleftherianos et al., 2007), disease resistance (Wilson et al., 2001), and more generally the evolution of life-history traits (Mcnamara et al., 2013), sexual conflicts (Steiger et al., 2012) and social life (Schmid-Hempel, 1998). A commonly used, low-cost, and fast method to measure individual immunocompetence in insects is to analyze the activity of the prophenoloxidase (PPO) and phenoloxidase (PO) system (Cerenius and Söderhäll, 2004). This non-specific immune defense is typically activated after wounding and/or parasite infection, and is known to play a central role in the melanization and encapsulation of parasites (Beckage, 2008). The standard laboratory process to estimate PPO and PO activities is fast and straightforward, making its use extremely popular among researchers. It consists of mixing insect hemolymph (or cuticular extracts) with L-DOPA and then spectrophotometrically follow the speed at which L-DOPA is enzymatically transformed into dopachrome (i.e. the speed at which the mix becomes darker). The resulting curve of absorption is then used to extract the values of PPO and PO activities for the given individual.

To date, a surprisingly large number of methods are routinely used to extract PPO and PO activities from spectrophotometer data. These methods involve either a single, a specific subset or all values of the reaction curve, and define PPO and PO activities either as the absorption value at a given time of the reaction curve, as the increase in absorption between two reaction times, or as the slope of the reaction curve during a presumed linear phase (fixed *V*max) defined by the user (Table 1). The fundamental differences between these methods and the absence of consensus on their use across laboratories however raise two crucial issues. First, estimating PPO and PO activities using different methods produce results with different units, range and biological meanings, which consequently hamper any direct comparisons among them. Second, each method is based on a calculation that can respond differently to the property of each curve, which can be a major problem for the reliability of the measurements across studies. Such an issue may result, for instance, from the saturation of the enzymatic reaction, the time windows arbitrarily fixed to calculate the mean slope of absorption curves across a data set and the noise

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Table 1
Overview of the methods used to calculate PPO/PO activities commonly found in the literature. Methods were clustered with respect to their underlying algorithm. We illustrated both the striking differences between the results given by each method and the difficulty to compare these values among each other by calculating mean PO activity in three large and independent data sets based on the European earwig *F. auricularia* (2050 absorption curves, D1), the ant *C. cursor* (192 curves, D2) and the mealworm beetle *T. molitor* (94 curves, D3). Abs_{tn} = Absorption at *n* minutes.

Type	Potential limitations	Method ID	Description	Mean PO activity			Example of references
				D1	D2	D3	
Time difference	• Sensitive to outliers	M1	Abs_{t15}–Abs_{t0}	0.094	0.100	0.421	Cotter et al. (2004)
	• Depends on the saturation of the chemical reaction	M2	Abs_{t10}–Abs_{t0}	0.042	0.063	2.261	Demuth et al. (2012)
		M3	Abs_{t20}–Abs_{t0}	0.148	0.131	6.084	Adamo (2004)
		M4	Abs_{t15}–Abs_{t5}	0.085	0.080	3.887	Srygley et al. (2009)
		M5	$Abs_{t16,200}$–Abs_{t0}	0.487	0.204	12.368	Mucklow and Ebert (2003)
Single point	• Sensitive to curves with a delayed increase	M6	Abs_{t30}	0.324	0.232	13.750	Dubovskiy et al. (2013)
	• Sensitive to noisy graphs	M7	Abs_{t20}	0.230	0.189	10.400	Reeson et al. (1998)
Highest value	• Sensitive to curves with a delayed increase • Sensitive to noise	M8	Maximum rate of reaction within 30 min	0.015	0.012	0.871	Rantala et al. (2002)
Fixed *V*max	• Sensitive to curves with high absorbance at beginning and/or a delayed increase	M9	Slope between 5 and 15 min	0.001	0.001	0.099	Siva-Jothy et al. (2008)

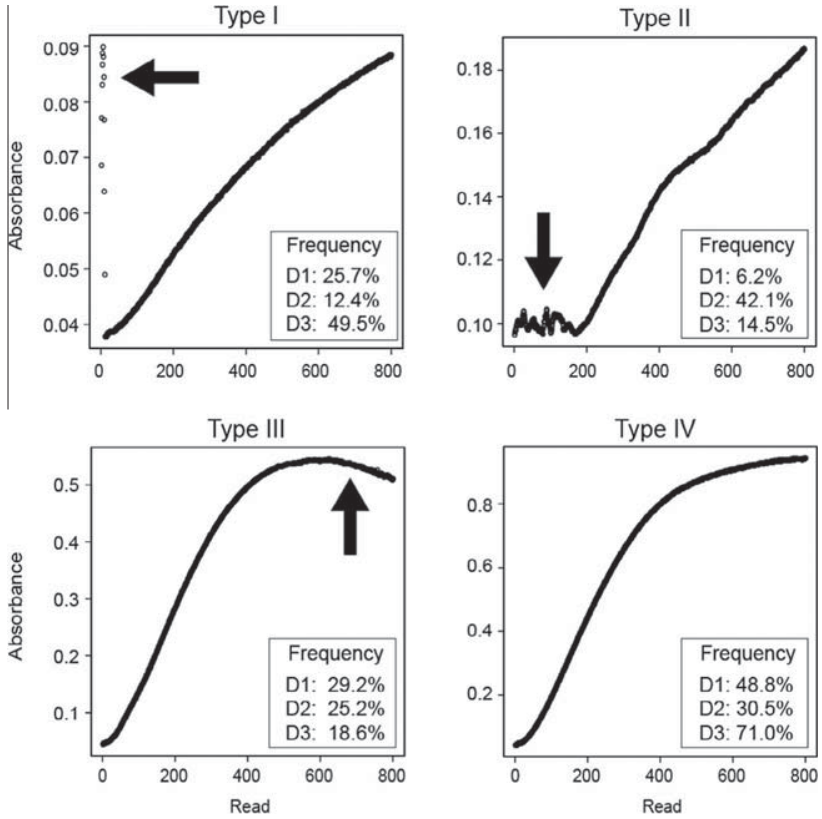

Fig. 1. Types of noise commonly found in PPO/PO curves and their frequency in three large and independent data sets. Curves had either (Type I) abnormal values at the beginning of the curve, (Type II) elongated asymptotic section at the beginning of the curve, (Type III) decreased values at the end of the curve or (Type IV) no apparent noise. Arrows highlight the location of the noise in the curve. The frequencies of each curve are reported (in the boxes) for three data sets containing (D1) 2050 curves measured in the European earwig *F. auricularia*, (D2) 192 curves in the ant *C. cursor* and (D3) 94 curves in the mealworm beetle *T. molitor*. Because the types of noise are non-mutually exclusive, the reported frequencies do not sum-up to 100% per data set.

frequently present in PPO and PO curves (Table 1 and Fig. 1, Wilson and Walker, 2010).

In this study, we demonstrate the importance to standardize the measurements of PPO and PO activities across studies, and present *PO-CALC*, a novel method to do so. Specifically, we (1) demonstrate that nine methods commonly used in the literature to extract PPO and PO activities provide different results when tested on the same data sets. We then (2) show that these different results at least partly rely on the sensitivity of each method to the noise regularly shaping PPO and PO absorption curves. Finally, we (3) present the R based program *PO-CALC*, which is a free, novel, simple and robust method to extract PPO and PO

activities from large data sets and (4) demonstrate its robustness and efficiency by comparing its results to the ones given by the nine methods described above.

2. Material and methods

2.1. Comparing PO activities using nine methods commonly found in the literature

To demonstrate the importance of standardizing PPO and PO measurements across studies, we calculated the mean PO activity in three large and independent data sets using nine methods commonly found in the literature. The three data sets included a total of 2050 absorption curves (820 PO and 1230 PPO curves) in the European earwig *Forficula auricularia* (Lisa K. Koch and Joel Meunier, Unpublished data), 192 PO absorption curves in the ant *Cataglyphis cursor* (kindly provided by Claire Tirard; University Paris VI, France; Unpublished data), and 94 PO absorption curves in the mealworm beetle *Tenebrio molitor* (kindly provided by Yannick Moret; University of Dijon, France; published in Zanchi et al., 2011). Note that for these data sets, the PPO and PO absorption curves have been obtained respectively with and without the addition of chymotrypsin into the hemolymph + L-DOPA mix. The nine methods selected to extract PPO and PO activities are the most common in the literature and encompass five methods calculating the difference of absorption between two times of the curve, two methods using a single point of the curve, one method using the highest value of the curve and finally one method using a fixed *V*max calculation (see details in Table 1).

2.2. Sensitivity of each method to the noise regularly found in absorption curves

We first quantified the frequency of three types of noise commonly known to shape PPO/PO absorption curves: Type I noises are characterized by abnormal values at the beginning of the curve, Type II by an elongated asymptotic section at the beginning of the curve, and Type III by decreased values at the end of the curve (details in Fig. 1). The origin of these noises is often unclear and probably multifactorial, reflecting for instance, delayed or incomplete mixing between hemolymph and L-DOPA, chemical reactions between unknown molecules contained in the hemolymph, and/or interferences of the laser with pieces of tissue of the insect body mistakenly transferred to the well plate.

We then investigated to what extent these three types of noise influence the calculation of PO activities by nine methods commonly found in the literature (Table 1). To this end, we used the large data set of 2050 PO absorption curves obtained in the European earwig *F. auricularia* (see above), in which we haphazardly picked up 25 curves with each type of noise and 25 curves with no apparent noise (i.e. a total of 100 curves). For each of these curves, we then extracted PO activities using individual *V*max calculation as a reference, and compared these values to the ones obtained with the nine methods presented in Table 1. For individual *V*max calculation, the slope (*V*max) of a linear regression conducted on the part of the graph visually showing the most decent linear dependency was extracted. It was used as a reference because it is considered as the most accurate (albeit time consuming when applied to large data sets) method to calculate enzymatic reactions (Wilson and Walker, 2010). The comparison was then done by conducting a series of linear regressions between the values obtained with individual *V*max calculation for each type of curve and the ones with the nine methods presented in Table 1 (i.e. a total of 40 regressions). From each of these regressions, we extracted (1) the coefficient of determination R^2 (thereafter called accuracy), which reflects the fit of the regression and thus the sensitivity of the method to the tested type of noise (1 = perfect fit, 0 = no fit), and then (2) the Fold change between the values obtained with individual *V*max calculation and the corresponding method (thereafter called comparability), which shows the degree to which the two sets of values differ and thus the comparability of the tested method (1 = high comparability, <1 or >1 = low comparability). Finally, we tested the robustness of *PO-CALC* (see below) by repeating the same analyses with the values obtained with this software.

2.3. Description of the PO-CALC algorithm

We developed a program, called *PO-CALC*, to address the important issues raised by the different methods used to measure PPO and PO activities across studies. This program automatically calculates *V*max from each reaction curve, which is the most accurate method to determine enzymatic activity (Wilson and Walker, 2010). It does so through four steps that can be briefly described as: (a) calculation of the slope of each data point contained in each absorption curve, (b) automatic identification of the linear part of each absorption curve, (c) robust calculation of *V*max by averaging the slopes of the data points contained in the linear part of the curve and (d) automatic removal of non-reliable *V*max calculations from the full data set. Note that *PO-CALC* has been designed and optimized for data sets including 800 reads per sample with 10 s interval, and that it requires a minimum of 400 reads per sample. To increase user friendliness, the R script (also available) defining *PO-CALC* was embedded into a Java based Graphical user interface (GUI). Java version 7 or higher are required.

The four steps involved in *PO-CALC* calculations are detailed below. To improve clarity, the names of the vectors created by the program are in *italics*, the names of the R commands are between “ ”, and the parameters that can be directly modified in the GUI have an asterisk. Default settings of the program have been determined by testing different settings of all parameters and using the combination that produced the most accurate results (data not shown).

2.3.1. Calculation of slope values for each data point

A polynomial model of sixth degree is applied to each curve using the R package polynom. Applying the polynomial model limits the influence of outliers in the curve on the resulting measurements. The polynomial function is derived to calculate slope values of each data point X. Note that even if polynomial model of fifth degree could be sufficient to analyze most curves, the implementation of sixth degree allowed *PO-CALC* to cover the relatively few curves affected by the highest (observed) level of noise.

2.3.2. Identification of the linear part of the absorption curve

Based on the slope values obtained from the above polynomial model, data points are classified either as High or Low slope. To this end, the change in absorption of each data point X within a 50 data point environment ($X - 5^*$ to $X + 45^*$) is determined. The point is set as High slope if this change is above 8.5%* of the overall change in absorption and Low slope otherwise. To locate the start of the linear section, the (X − 30) − X environment of each data point is automatically screened for the number of High and Low slope entries. If more than 15* entries are classified as High, the data points are transferred to a new vector named *clean_highslope*. This transfer stops when 15* out of the next 40 slope entries were Low. Note that to define the default settings, parameters were systematically changed and the combination of parameters that produced the most accurate results on 75 graphs from thee different PPO/PO data sets were chosen (data not shown).

2.3.3. Robust calculation of Vmax

The mean of all entries saved in the vector *clean_highslope* is calculated and saved in the vector *vmax_values*.

2.3.4. Removal of non-reliable Vmax values from the full data set

Three different filters are applied to identify and remove *V*max values that are based on calculations characterized by a low reliability. (1) The accuracy of the polynomial model was tested using the "summary()" function. If two or more out of six coefficients (polynomial of the sixth degree) do not make a significant contribution to the fitting of the model to the data, the respective entry in *vmax_values* is set to NA. (2) If the first 16.6% of the data points of a graph were associated with negative slope values, the absorption curve is considered to be an artefact and the respective *vmax_values* are set to NA. (3) As *V*max calculation based on few values tend to be over- or underestimated, *V*max based on less than 60* data points were set to NA.

3. Results and discussion

The three types of noise varied in their frequency across data sets. The frequency of Type I noise ranged between 12.4% and 49.5%, Type II between 6.2% and 42.1%, Type III between 18.6% and 29.2, whereas the proportion of curves without apparent noise ranged from 30.5% to 71.0% (Fig. 1). The nine methods commonly used in the literature to extract PO activities provided results varying up to 13,000% when tested on the same data sets (Table 1). In particular, the nine methods provided mean PO activities ranging from 0.001 to 0.487 when tested in earwigs, from 0.001 to 0.232 when tested in ants and from 0.099 to 13.75 when tested in beetles (Table 1). Importantly, the nine methods ordered differently the mean PO activities among the data sets, with two methods reporting higher activities in earwigs than ants, six methods reporting higher activities in ants than earwigs, and one method reporting similar activities between the two data sets (Table 1). These method-specific differences at least partly reflect the sensitivity of each method to the different types of noise present in the absorption curves (Table 2), as revealed by the mean accuracy (mean R^2) of each method, which ranged from 0.46 to 0.88, and their Fold change which had values between 1.15 and 159.65 (Table 2). Hence, the extreme variability of the results provided by the different methods, its consequences on data interpretation, and the method-specific effects of spectrophotometer noise emphasizes the necessity of a novel, standardized and reliable method to extract *V*max values from PPO/PO graphs.

Table 2
Coefficients of determination (R^2) and accuracy between the results from individual *V*max calculation and each method for PO curves with different types of noise. Curves had either (Type I) abnormal values at the beginning of the curve, (Type II) elongated asymptotic section at the beginning of the curve, (Type III) decreased values at the end of the curve or (Type IV) no apparent noise. Methods are sorted decreasingly with respect to the mean R^2. The two methods with the fittest coefficient of determination and Fold change value per type of curve are in bold.

Method ID	Type of absorption curves				Mean R^2	Comparability
	I	II	III	IV		
PO-CALC	**0.99**	**0.89**	**0.94**	**0.99**	**0.95**	**1.22**
M6	**0.99**	0.79	0.77	**0.99**	**0.88**	159.65
M7	0.70	0.62	0.93	0.98	0.88	117.51
M3	0.86	0.26	0.88	0.98	0.75	84.99
M5	0.88	**0.96**	0.24	0.69	0.69	191.76
M8	0.23	0.62	**0.96**	0.90	0.67	7.92
M9	0.65	0.20	0.85	0.97	0.67	**−1.15**
M1	0.66	0.10	0.82	0.96	0.64	57.85
M4	0.06	0.10	0.80	0.97	0.48	48.8
M2	0.18	0.04	0.66	0.94	0.46	33.96

Our results demonstrate that *PO-CALC* offers this novel, standardized and reliable method. First, the measures it extracted for PPO and PO activities were less sensitive to the different types of noise commonly found in absorption curves, as reflected by a mean accuracy of 0.95, a Fold change of only 1.22, and the fact that it achieves the highest or second highest accuracy (R^2) for every type of curve (Table 2). Second, *PO-CALC* offers a unique approach that reduces the impact of fluctuations or single outlier data points within the curve by applying a polynomial model prior to the identification of slope values. Finally, *PO-CALC* automatically determines the linear section of each graph individually and then uses this specific subset for *V*max calculation, which does not require a fastidious and sometime not objective determination of time windows to run the calculations (as in method 9, Table 1).

Multiple factors may explain why researchers used a variety of different methods to extract PPO and PO activities from spectrophotometer data. A first one is that they have a time constraint to test alternative methods to the one they have already established in the laboratory. A second one is that researchers might be tempted to use the method offered by the software implemented in their spectrophotometer, which rely on calculations that differs between companies. A third hypothesis is that researchers might be unaware of the potential limitations of the calculation underlying the methods they are using. Finally, closed source software packages might not have been considered by researchers due to license restrictions.

One option to circumvent these problems is to generalize the use of one simple, robust, and free method across studies on PPO and PO measurements. *PO-CALC* offers this possibility: It is easy to implement due to its user-friendly interface (also available as R script), it is free, it can work on the raw data provided by every type of spectrophotometer, and it was thoroughly tested against other calculation methods. Furthermore, its standardized and automatic calculations offer a novel and important tool to compare measurements across experiments. Finally, *PO-CALC* can be used to calculate the *V*max of other enzymatic reactions, given that their reaction kinetic is comparable to the PO and PPO ones, and that the strengths of *PO-CALC* are thoroughly tested for these reactions (e.g. using the same comparative approach than the one presented in this study). Overall, *PO-CALC* has the potential to increase the significance and general validity of studies estimating invertebrate immunity and by doing that, to contribute to a broad range of research questions located in the intersection of evolutionary biology, ecology and physiology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2015.02.015.

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Feces production as a form of social immunity in an insect with facultative maternal care

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Abstract

Background: Social animals have the unique capability of mounting social defenses against pathogens. Over the last decades, social immunity has been extensively studied in species with obligatory and permanent forms of social life. However, its occurrence in less derived social systems and thus its role in the early evolution of group-living remains unclear. Here, we investigated whether lining nests with feces is a form of social immunity against microbial growth in the European earwig *Forficula auricularia*, an insect with temporary family life and facultative maternal care.

Results: Using a total of 415 inhibition zone assays, we showed that earwig feces inhibit the growth of two GRAM+ bacteria, two fungi, but not of a GRAM- bacteria. These inhibitions did not result from the consumed food or the nesting environment. We then demonstrated that the antimicrobial activity against fungus was higher in offspring than maternal feces, but that this difference was absent against bacteria. Finally, we showed that family interactions inhibited the antibacterial activity of maternal feces against one of the two GRAM+ bacteria, whereas it had no effect on the one of nymphal feces. By contrast, antifungal activities of the feces were independent of mother-offspring interactions.

Conclusion: These results demonstrate that social immunity occurs in a species with simple and facultative social life, and thus shed light on the general importance of this process in the evolution of group-living. These results also emphasize that defecation can be under selection for other life-history traits than simple waste disposal.

Keywords: Social immunity, Family life, Feces, Precocial, Insect, Earwig

Background

One of the major costs of group-living is its inherent risk of pathogen infection for group members [1-3]. While solitary species can only use personal immune responses to fight against infections, group-living species also possess the unique capability of mounting collective immune defenses, a phenomenon called social immunity [2,4]. Over the last two decades, a growing number of studies showed that multiple forms of social immunity can be expressed in species with permanent and obligatory social life, such as eusocial insects (reviewed in [2]). These studies were of great interest for the development of research on social immunity in insects, because they demonstrated that the high risks of pathogen infection associated with obligatory and complex forms of social life were likely to select for the emergence of collective defenses against pathogens [2,4]. However, they were of limited relevance to understand whether social immunity only emerged in eusocial systems and therefore represents a secondary trait derived from eusociality, or whether it also occurs in less derived forms of group-living and thus possibly plays a central role in the early evolution of group living organisms [2,4].

One method to address this issue is to investigate the occurrence of social immunity in species with temporary and facultative group-living. This is the case of species with family life, which represents a common form of group-living in insects [5,6], can be temporary and facultative such as in precocial species [7,8] and is generally considered as a major step in the evolutionary route to eusocial systems [6,9]. In insects, family life is broadly associated with the expression of care to the eggs and/or juveniles, such as protection against predators, clutch displacement and food provisioning [5,10]. Family life may also include forms of social immunity before egg hatching. For instance, parents groom their eggs to

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prevent the development of fungal spores in the European earwig *Forficula auricularia* [11], apply bacteria with antifungal properties to brood cell prior to oviposition in the European beewolf *Philanthus triangulum* [12], coat their nest with antimicrobial secretions in the housefly *Musca domestica* [13] or prophylactically avoid nest sites with high microbial pressure in the burying beetle *Nicrophorus vespilloides* [14]. Although pre-hatching forms of social immunity have been well studied in insects, surprisingly little is known about the nature and occurrence of the post-hatching ones (see e.g. in vertebrates [15,16]). Only recent studies showed that parental anal exudates and larval secretions exhibit antimicrobial properties in the burying beetles [17-19]. In this species, however, larvae feed on the carcass serving as nesting habitat, so that these antimicrobial mechanisms could also reflect evolutionary responses to competition with microbes over food access and/or to the extraordinarily high microbial pressure in this specific habitat.

In this study, we investigated whether social immunity occurs in the form of the production of feces with antimicrobial activity in the European earwig *F. auricularia*, an insect with temporary and facultative family life. In this species, mothers provide care to their offspring in soil burrows for several months, during which all family members - once hatched - line ground and walls with their feces pellets [8,20-23]. Earwig maternal care can take multiple forms, such as egg and juveniles (called nymphs) attendance and food provisioning through regurgitation, which have been shown to enhance offspring fitness [8,11,24,25]. Nevertheless, nymph survival does not require maternal care, as nymphs are mobile at hatching and can forage for themselves [8,26]. Here, we first tested whether (1) earwig feces provides a form of social immunity by inhibiting the development of bacteria and fungus into the nest, and determined whether these effects were independent of the consumed food and nesting material. We then investigated whether (2) antimicrobial activity was stronger in maternal compared to nymphal feces, as expected under the assumption that it reflects a post-hatching form of maternal care. Finally, we tested whether (3) the antimicrobial activity of feces is a socially-mediated trait that is triggered or inhibited by experiencing mother-offspring interactions [17]. If antimicrobial properties are induced by mother-offspring interactions, we predict that the feces produced by isolated individuals show lower antimicrobial activities. Conversely, we predict higher antimicrobial activities in feces produced by the isolated individuals if the costs of producing antimicrobial agents in the feces entail a mother-offspring conflict, in which each party tries to reduce its own investment into the production of antimicrobial components while benefiting from that of the other.

Methods

Insect rearing and feces collection

We collected feces pellets in 17 *F. auricularia* families composed of one mother and 36.11 ± 15.8 (mean ± SD) nymphs. These mothers were the first laboratory-born generation of individuals field sampled in 2012 in Dolcedo, Italy, and then maintained under standard laboratory conditions (rearing details in [27]). To determine whether the occurrence of mother-offspring interactions influences the antimicrobial properties of maternal and nymphal feces, the 17 families were randomly distributed among two groups at egg hatching. In the first group, we experimentally prevented mother-offspring interactions by separating mothers from their clutch of nymphs one day after egg hatching (Isolation group, n = 10). By contrast, mothers in the second group were separated from their nymphs ten days after egg hatching (Family group, n = 7). These separations were done by transferring the mother and the clutch of nymphs to two new petri dishes. At day 10, mothers and groups of nymphs from family groups were separated and transferred into two new petri dishes, in which they were maintained until feces collection at day 13 (first developmental instars). This manipulation was also done on the individuals from the isolation groups to standardize the experimental process. The transfer and three day delay between separation and feces collection ensured that the collected feces was relatively fresh and in large enough quantity to conduct the radial diffusion assays.

Individuals received *ad libitum* standardized food (for food composition, see [27]) from day 1 to day 9, and *ad libitum* green-colored pollen (Hochland Bio-Blütenpollen by Hoyer; Food die by DEKO BACK) from day 10 to day 12. Under these conditions, orphaning does not affect nymph quality in terms of developmental time and survival rate (Koch LK and Meunier J, unpublished data). The use of colored pollen is common in earwig experiments (e.g. [21,25,28]) and was used here to disentangle feces pellets from sand grains in the rearing containers. At day 13, all (colored) feces pellets present in each petri dish were collected using a sterile 10 μl pipette tip. For each petri dish, the total amount of collected pellets was weighed to the nearest 0.1 μg (Pescale), then suspended in 500 μl sterile NaCl solution (0.9%) and finally stored at 4°C. This feces solution was used 2.6 ± 1.5 days (mean ± SD) later to conduct the radial diffusion assays (see below). All petri dishes (diameters 10 and 5 cm before and after separation, respectively) contained humid sand as substrate and a plastic shelter as a nest. They were maintained in a climate chamber at 60% humidity, constant 20°C and 10:14 h light/dark cycle during the course of the experiment.

Radial diffusion assays

We tested the antimicrobial properties of maternal and nymphal feces using a total of 170 radial diffusion assays against two GRAM+ bacteria, one GRAM- bacteria, and two fungi species (see details below). Radial diffusion assays were conducted in petri dishes (diameter 10 cm) filled with PDA (Potato Dextrose Agar, 70139, SIGMA-ALDRICH) covered with a solution of 10^9 bacteria or spores/ml. Four samples were tested per plate. To this end, each fourth of a PDA plate received a blank disc (antimicrobial susceptibility test discs, OXOID) in its center, on which 10 μl of feces solution was preliminary applied. The same process was used to conduct a total of 245 controls (49 per microbial species), in which we tested whether growth inhibition could result from the NaCl solution used to dilute the feces (n = 15/species), the food eaten by the tested individuals (10 mg of colored pollen pellets suspended in 1 ml NaCl solution, n = 15/species; 240 mg of standardized food source suspended in 1 ml NaCl solution, n = 4/species) or the sand on which feces has been released (50 mg of sand suspended in 1 ml NaCl solution; n = 15/species). After inoculation, each plate was incubated at 36°C/24 h for bacteria and at 20°C/48 h or 20°C/72 h for the fungus (for *Saccharomyces cerevisiae* and *Aspergillus niger*, respectively). At the end of the incubation, the zone of clearance (diameter from one edge of the zone of inhibition to the other) was measured three times per sample and then averaged to give one mean value called antimicrobial activity.

The radial diffusion assays were conducted against five microbial species covering a spectrum of groups that have the capability to grow into earwig burrows. First, we used *Staphylococcus aureus* (NCIMB 9518), which is a GRAM+ bacteria known to secrete a range of enzymes and toxins associated with several diseases in vertebrates and invertebrates [29]. Second, *Bacillus subtilis* (ATCC 6633) is another GRAM+ bacteria, which is a facultative pathogen commonly found in the soil [29]. Third, *Escherichia coli* (ATCC 25922) is a GRAM- bacteria typically found in the intestinal tracts of mammals and insects [29]. Fourth, *Saccharomyces cerevisiae* (ATCC 2601) is a fungus known to cause lethal infections in invertebrates [29]. Finally, *Aspergillus niger* (wild type strain) is a fungus growing on rotten plant material that can be an opportunistic pathogen [29].

Statistical analyses

We first tested the effect of feces producer (mother or nymphs), family life (isolation or family group) and their interaction on the log-transformed amount of feces produced between day 10 and 13 (i.e. the amount of diluted feces) using a linear model. Because inhibition zones do not follow normal distributions and include a substantial number of zeros across the radial diffusion assays (see results), the significance of the effects of feces producer and family life on antimicrobial activity were then tested using a series of randomized analysis of variance (randomized ANOVA; [30]). This non-parametric method allows estimating the significance of a factor (i.e. calculate p-values) by running a series of 10'000 ANOVA, in which the response variable (i.e. antimicrobial activity or antimicrobial activity per mg of feces) is permuted across the explanatory factors (i.e. feces producer and family life). Finally, we conducted pairwise comparisons between the antimicrobial activities of the controls (pooled) and the ones of the maternal or the nymphal feces using Mann–Whitney rank tests, in which the significance level $\alpha = 0.05$ was adjusted for multiple testing to $\alpha = 0.025$ using Bonferonni correction. All statistical analyses were conducted using the software R v3.1.1 (http://www.r-project.org). The R script to conduct randomized ANOVA is available on demand.

Results

Each mother produced on average 13.06 ± 2.34 mg (mean ± SE) of feces between day 10 and day 13. This quantity was smaller than the 180.63 ± 18.88 mg of feces produced by the clutch of nymphs during the same period of time (Likelihood Ratio (LR) $\chi^2_1 = 252.72$, $P < 0.0001$). The total amount of feces produced over three days was independent of family isolation (LR $\chi^2_1 = 0.97$, $P = 0.324$), or of an interaction between family isolation and feces producer (LR $\chi^2_1 = 1.12$, $P = 0.290$).

Inhibition zones were found in 25 (73.5%) assays against *B. subtilis*, 10 (29.4%) against *S. aureus*, 19 (55.9%) against *S. cerevisiae*, 17 (50.0%) against *A. niger*, but none (0.0%) against *E. coli.* Maternal feces inhibited the growth of at least one microbial species in 13 (76.5%) of the 17 tested families, while nymphal feces had inhibition effects in every sample from the 17 (100%) families. None of the controls (NaCl, pollen, standardized food and sand) showed antimicrobial activity in any of the 245 assays (Figure 1).

The antimicrobial activity of maternal and nymphal feces produced over three days depended on the feces producer and the microbial species, but not on the occurrence of mother-offspring interactions (Table 1a, Figure 1). Specifically, antimicrobial activities against *A. niger and S. cerevisae* were lower in maternal compared to nymphal feces, whereas antimicrobial activities against *B. subtilis* and *S. aureus* were independent of feces producer (Table 1a). Except against *E. coli*, each type of feces showed higher antimicrobial activity than the controls (Table 2, Figure 1). The general antibacterial activity of nymphal feces against *S. aureus* was mostly driven by three points in the data set (Figure 1). If these three points were excluded, the resulting mean antibacterial activity of

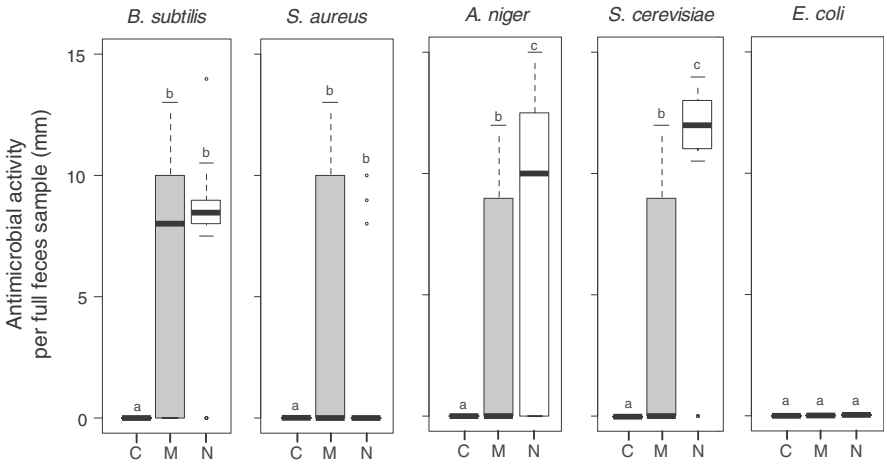

Figure 1 Antimicrobial activities of controls (C), maternal (M, grey) and nymphal (N, white) feces. Controls combine assays with NaCl, Pollen, Food and Sand. Boxplots depict median (bold bar) and interquartile range (light bar), with outlying values (circles) and whiskers extending to 1.5 times the interquartile range. Different letters indicate P < 0.005.

nymphal feces against *S. aureus* would become null and thus smaller than the one of maternal feces (Mann–Whitney test, W = 168, p = 0.008) and similar to the controls (Figure 1).

In line with the prediction that antifungal components are more concentrated in maternal than nymphal feces, we found that the antimicrobial activities per mg of feces against *B. subtilis and S. aureus* were larger in maternal compared to nymphal feces (Table 1b, Figure 2). By contrast, feces producer did not influence such activity against *S. cerevisae* (Table 1b, Figure 2). Overall, the occurrence of mother-offspring interactions did not shape the antimicrobial activities per mg of feces against *B. subtilis*, *S. aureus* and *S. cerevisae* (Table 1b, Figure 2). However, it interacted with feces producer to shape the antimicrobial activity per mg of feces against *A. niger* (Table 1b, Figure 2). Specifically, the presence of mother-offspring interactions canceled the antimicrobial activity of maternal feces (Mann–Whitney rank test; W = 52.5, P = 0.040) but had no effect on the one of nymphal feces (Figure 2, W = 38, P = 0.807). Note that this interaction was only marginally non-significant when analyzing the overall antimicrobial activity of maternal feces produced over three days (Table 1a).

There was no family effect on the antimicrobial activities of nymphal and maternal feces (Table 3). Across microbial species, antimicrobial activities were comparable (present or absent) between maternal and nymphal feces in 51.4% of the families, a value that was not significantly different from a random distribution (Binomial test against 50%, P = 0.904). Note that the four microbial species (excluding *E. coli*) did not influence the proportion of families with comparable antimicrobial activities between maternal and nymphal feces (i.e. both present plus both absent *versus* present in only one type; Pearson's Chi-squares test, $\chi^2 = 3.0$, df = 3, P = 0.391).

Discussion

Gaining a better understanding of the evolution of the multiple forms of group-living requires insights into the

Table 1 Influences of feces producer and mother-offspring interactions on antimicrobial activities (a) per full sample and (b) per mg of feces

	B. subtilis	*S. aureus*	*A. niger*	*S. cerevisiae*
(a) Activity per full feces sample				
Feces producer (FP)	P = 0.440	P = 0.098	**P = 0.005**	**P <0.0001**
Mother-offspring interactions (MO)	P = 0.808	P = 0.104	P = 0.342	P = 0.051
FP : MO	P = 0.553	P = 0.934	P = 0.068	P = 0.215
(b) Activity per mg of feces				
Feces producer (FP)	**P <0.0001**	**P = 0.008**	P = 0.095	P = 0.080
Mother-offspring interactions (MO)	P = 0.813	P = 0.575	P = 0.052	P = 0.078
FP : MO	P = 0.812	P = 0.731	**P = 0.037**	P = 0.089

Feces producers were either the mother or the nymphs. P-values were obtained from randomized ANOVAs and the significant ones are in bold.

Table 2 Comparisons between inhibition zones generated by the controls and the total amount of either maternal or nymphal feces

	B. subtilis		*S. aureus*		*A. niger*		*S. cerevisiae*	
	W	*P*	*W*	*P*	*W*	*P*	*W*	*P*
Maternal feces	686	**<0.0001**	588	**<0.0001**	539	**<0.0001**	539	**<0.0001**
Nymphal feces	759.5	**<0.0001**	490	**0.0028**	710.5	**<0.0001**	759.5	**<0.0001**

Statistical values were obtained from Mann–Whitney tests. Significant P-values are in bold. All p-values remain significant after correcting for multiple testing.

mechanisms that help individuals to limit the inherent risk of infection. Here, we demonstrate that lining nests with feces inhibits microbial development in the European earwig. Specifically, earwig feces showed antimicrobial activities against two GRAM+ bacteria (*B. subtilis* and *S. aureus*) and two fungi (*A. niger* and *S. cerevisiae*). These antimicrobial properties are likely to provide immune benefits to earwig family members, as many microbial entomopathogens have the capability to grow under the underground conditions provided by insect nests (e.g. [1,31]), several of them are known to frequently attack earwig nests [32-35], and a recent study showed that even the development of non-entomopathogenic fungus into the nest comes with detrimental effects on earwig fitness [11]. Together with the fact that earwig nymphs produce more feces when encountering related compared to unrelated conspecific juveniles [21], these results thus support that feces production at least partly reflects a kin-triggered form of social immunity.

The maintenance of feces in the nest is a poorly studied phenomenon in eusocial insects [36,37], in which colony members are generally assumed to evacuate feces into specific nest chambers to prevent microbial development in the colony (reviewed in [2,38]). This phenomenon has nevertheless been reported in two non-eusocial insects exclusively feeding on their nesting material, the wood cockroach *Cryptocercus punctulatus* and the burying beetle *N. vespilloides* [14,19,39], for which the use of anal exudates (and their antimicrobial activity) into the nest has been proposed to have at least partially evolved to limit competition with microbes over food access [19,40].

Our study shows that the total amount of feces produced by mothers over three days did not exhibit higher antimicrobial activities than the one produced by nymphs, revealing that feces antimicrobial activity is not a simple form of post-hatching maternal care. Instead, we show that nymphs contributed more to antifungal nest protection than mothers, mostly due to their overall larger production of feces (each mg of nymphal feces exhibited similar antifungal activity than each mg of maternal feces). This higher feces production also allowed nymphs to compensate for the lower intrinsic antibacterial activity of their feces (activity per mg of feces) against GRAM+ bacteria, thus exhibiting an overall antimicrobial activity comparable to the one of maternal feces. This age-specific effect on the antimicrobial activity per mg of feces suggests differences in composition between nymphal and

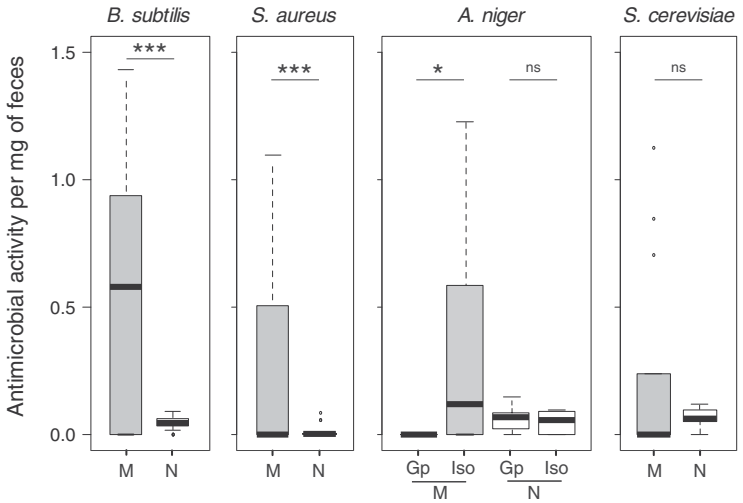

Figure 2 Antimicrobial activities per mg of maternal (M, grey) and nymphal (N, white) feces. When reported, feces producers were either maintained in family groups (Gp) or isolated (Iso) before feces collection. Boxplots depict median (bold bar) and interquartile range (light bar), with outlying values (circles) and whiskers extending to 1.5 times the interquartile range. ***P < 0.001; *P < 0.05; ^{ns}P > 0.05.

Table 3 Expression of feces antimicrobial activity per family

Antimicrobial activity in		*B. subtilis*	*S. aureus*	*A. niger*	*S. cerevisiae*	*E. coli*
Maternal feces	Nymphal feces					
Yes	Yes	8	2	3	5	0
No	No	0	9	3	3	17
Yes	No	3	5	2	0	0
No	Yes	6	1	9	9	0

For each of the five microbial species, we reported the number of family in which an antimicrobial activity was found in both maternal and nymphal feces, in none of them or in either maternal or nymphal feces.

maternal feces. Feces compositions could differ in terms of quantity and/or quality of residual compounds of their personal immunity, which are known to be present in the feces and to become stronger with aging in other insect species [41-45]. Another discrepancy in feces composition could result from differing hindgut flora of mothers and nymphs. The insects gut includes a great variety of symbiotic microorganisms that are crucial for growth and protection against infections [46-48], but that also change with aging [43,49,50]. Finally, nymphal and maternal feces could vary in terms of chemical products released during defecation. For instance, earwigs possess a pygidial gland on their abdomen that releases chemicals with antimicrobial properties [51]. Disentangling among these three non-mutually exclusive hypotheses will be addressed in further studies by investigating the presence of immune components and antimicrobial chemicals inside the feces, as well as by characterizing earwig gut flora.

We found that the antimicrobial activity of maternal feces depended on preliminary interactions with their nymphs. Specifically, family interactions inhibited the antimicrobial activity of maternal feces against *S. cerevisiae*, whereas they had no effect on the one of nymphal feces. This latter result contrasts with the one found in the burying beetle *N. vespilloides*, in which the absence of tending parents lowered the level of antibacterial activity in larvae exudates [17]. In earwigs, our result first reveals that the presence and/or quantity of the compounds mediating the antimicrobial activity of maternal feces against *S. cerevisiae* are socially-dependent. More generally, it suggests that mothers can adapt their investment into such a form of social immunity to the investment expressed by their nymphs. Assuming that investment into social immunity is energetically costly (see e.g. [52]), such maternal strategy could be adaptive and allow mothers to re-allocate their energy into other important life-history traits, such as forms of care and future reproduction [8,27]. Nevertheless, the effect of family life on feces antimicrobial activities was absent with the four other tested microbial species, indicating that the compounds mediating this activity are fixed during the period of family life. These compounds do not come from the environment, as there was no antimicrobial activity in the food consumed by the individuals and in the sand covering the rearing containers.

A somewhat surprising result of our study was the large number of feces samples with no antimicrobial activity. These negative assays are unlikely to reflect a problem in our methodology, as radial diffusion assay is a standard procedure that has been commonly used to test antimicrobial activities in other insect species (e.g. [18,36]). They are also unlikely to reflect that feces antimicrobial activity is a family-trait only expressed in a limited number of families, since we showed that the occurrence (or absence) of feces antimicrobial activities was not necessarily the same between nymphs and mothers from the same family. Conversely, our result could reflect a form of specificity in the immune responses mediated by the feces, which is in line with the fact that almost every feces sample inhibited the growth of at least one of the tested microbes. Another explanation could be that feces producers need some cues to switch on antimicrobial activity in their feces. These cues are unlikely to come from our standardized rearing environment, but might reflect that some field sampled mothers have been naturally exposed to pathogens prior sampling, and that such exposure affected the immunity of their own descendants through transgenerational immune priming [53]. However, the occurrence of transgenerational immune priming remains to be tested in *F. auricularia*.

Although earwig feces showed antimicrobial activity against the two tested GRAM+ bacteria, this activity was absent against the GRAM- bacteria *E. coli*. This lack of activity against *E. coli* has been reported in the antimicrobial secretions of other insect, such as the burying beetle *N. vespilloides* [18]. It may reflect either (1) higher physiological costs of mounting antimicrobial protection against GRAM- bacteria [41], (2) low selection pressure to mount defenses against GRAM- bacteria, e.g. because they are not present in their natural habitat or are important symbiotic organisms in the gut flora (but see [49]), or (3) specific resistance of the tested bacterial strain against the antimicrobial compounds present in earwig feces. Further studies should address this issue.

Conclusion

Overall, we demonstrate that social immunity in the form of lining nest with antimicrobial compounds can emerge and persist in species with primitive forms of group-living. Mounting collective defenses against microbial development could therefore be a widespread phenomenon across social systems and an important one in the early evolution of social life, as it does not require that individuals live in permanent and obligatory groups, and/or that group members compete with microbes for access to nest material as a food source.

Interestingly, these results also emphasize that defecation does not only reflect individual needs of waste disposal, but can be under selection for its importance in other crucial life-history traits [19,38,39].

Availability of supporting data

The data set supporting the results of this article is available in the DRYAD repository, http://doi:10.5061/dryad.9p31r [54].

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JMCD, MK and JM conceived the experiments; JMCD performed the experiment; JM performed the statistical analysis; MP provided lab facilities and microbial strains; JM wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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SHORT COMMUNICATION

Survival after pathogen exposure in group-living insects: don't forget the stress of social isolation!

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Keywords:

collective immunity;
earwig;
insect;
Metarhizium;
social deprivation.

Abstract

A major cost of group-living is its inherent risk of pathogen infection. To limit this risk, many group-living animals have developed the capability to prophylactically boost their immune system in the presence of group members and/or to mount collective defences against pathogens. These two phenomena, called density-dependent prophylaxis and social immunity, respectively, are often used to explain why, in group-living species, individuals survive better in groups than in isolation. However, this survival difference may also reflect an alternative and often overlooked process: a cost of social isolation on individuals' capability to fight against infections. Here, we disentangled the effects of density-dependent prophylaxis, social immunity and stress of social isolation on the survival after pathogen exposure in group-living adults of the European earwig *Forficula auricularia*. By manipulating the presence of group members both before and after pathogen exposure, we demonstrated that the cost of being isolated after infection, but not the benefits of social immunity or density-dependent prophylaxis, explained the survival of females. Specifically, females kept constantly in groups or constantly isolated had higher survival rates than females that were first in groups and then isolated after infection. Our results also showed that this cost of social isolation was absent in males and that social isolation did not reduce the survival of noninfected individuals. Overall, this study gives a new perspective on the role of pathogens in social evolution, as it suggests that an apparently nonadaptive, personal immune process may promote the maintenance of group-living under pathogenic environments.

Introduction

Group-living is a common phenomenon in nature, which ranges from temporary aggregations of conspecifics to permanent colonies with complex social organizations (Wilson, 1971). The ecological success of group-living species generally relies on the fitness benefits that social interactions provide to group members, such as enhanced foraging capability, facilitated access to mating partners and better protection against predators (Wilson, 1971; Krause & Ruxton, 2002). However, the frequent and tight behavioural interactions expressed by group members may also entail major fitness costs, as they typically favour the transmission of pathogens between individuals (Stroeymeyt *et al.*, 2014; Mccabe *et al.*, 2015; Theis *et al.*, 2015). The evolution of social life is therefore often associated with the development of mechanisms that improve the capability of group members to fight against this high pathogenic threat.

To limit this inherent risk of pathogen infection, many group-living animals have developed the unique capabilities to prophylactically increase their effort into personal immunity in the presence of conspecifics (called density-dependent prophylaxis or DDP; Wilson & Reeson, 1998) and/or to express collective defences against pathogens (called social immunity; Schmid-

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Hempel, 1998; Cremer *et al.*, 2007; Cotter & Kilner, 2010b; Meunier, 2015). DDP is often found in insects with simple forms of group-living. In these species, the presence of group members or a simple increase in group size usually entails higher investments into personal immune functions that enhance the survival of individuals exposed to pathogens (reviewed in Wilson & Cotter, 2009). On the other hand, social immunity is frequent in species with complex forms of group-living, such as eusocial insects (Pie *et al.*, 2005). In multiple species of ants, termites and bees, workers are known to remove corpses from the nest, to use antimicrobial substances as nest material and/or to express allogrooming behaviours to reduce the parasite load of group members (Cremer *et al.*, 2007; Meunier, 2015). Notably, a growing number of studies suggest that social immunity can also occur in species with facultative and temporary forms of social interactions, such as the ones expressed during early family life (Cotter & Kilner, 2010a,b; Diehl *et al.*, 2015; Meunier, 2015).

Over the last decades, the growing number of studies on DDP and social immunity emphasized that contact to group members may favour the survival of pathogen-exposed individuals in group-living species and that such benefits could promote the evolution of social life under pathogen pressure (Cremer *et al.*, 2007; Wilson-Rich *et al.*, 2009; Cotter & Kilner, 2010a; Meunier, 2015). However, it remains surprisingly unknown whether the absence of group members may also actively reduce (i.e. not only through the absence of benefits) an individual's capability to fight against an infection and thus may help promoting the maintenance of social life. Yet, many results showing that pathogen-exposed individuals survive better in groups than in isolation (e.g. Rosengaus *et al.*, 1998; Hughes *et al.*, 2002) are not only consistent with an effect of social immunity, but also with a cost of a sudden isolation on individual's survival after pathogen infections. Moreover, modifying a social environment is a well-known source of stress in group-living species, which often entails behavioural, physiological and/or neurobiological alterations affecting an individuals' lifespan (Hawkley & Cacioppo, 2003; Hawkley *et al.*, 2015; Koto *et al.*, 2015).

Here, we investigated whether the benefits of DDP, the benefits of social immunity (through interactions with group members) or the cost of social isolation determined the survival of pathogen-exposed adults in the European earwig *Forficula auricularia.* In this insect species, adults form groups with a size range from pairs to several dozens of individuals (Costa, 2006; Suckling *et al.*, 2006), in which they express social behaviours such as allogrooming (Weiß *et al.*, 2014) – a common mediator of social immunity (Reber *et al.*, 2011). Our experiment consisted in monitoring the survival rate of group-living female and male adults that were exposed either to the common entomophagous fungus *Metarhizium brunneum* or to a control solution. To disentangle the effects of DDP, social immunity and isolation stress, these individuals were either (a) maintained in their original groups after pathogen exposure (constant group-living), (b) isolated just after pathogen exposure (sudden isolation), or (c) maintained in isolation both before and after pathogen exposure (constant isolation). If adults' capability to fight against pathogen infection is solely driven by DDP, we predict that pathogen-exposed earwigs exhibit a higher survival rate in both the constant group-living and the sudden isolation (assuming that the effects of DDP last long enough after isolation) compared to the constant isolation treatments, while the two first treatments should lead to equally high survival rates. Conversely, if the presence of group members helps adult earwigs to limit pathogen infection only through social immunity, we predict that pathogen-exposed individuals survive better overall when kept in groups than in isolation after infection. Finally, if a stress of social isolation alters earwigs' capability to fight against pathogens, we formulated two predictions. If this stress is transient, we predict comparably high survival rates in the pathogen-exposed individuals constantly maintained in groups and in isolation, while their survival rates should be higher than the one of the suddenly isolated individuals. If this stress is cumulative over time, we expect that pathogen-exposed individuals survive better when maintained in groups, less well when suddenly isolated and the least well when constantly isolated.

Materials and methods

The *F. auricularia* adults used in this study were the first generation of a laboratory-born population field-sampled in 2013 in Mainz, Germany. All animals were bred under the standard laboratory conditions detailed in Weiß *et al.* (2014). The pre-exposure treatments took place two months after adult emergence, by transferring 359 virgin adults to Petri dishes either alone (N isolated females = 36, N isolated males = 35) or in experimental groups of four unrelated individuals of the same sex (N groups of females = 39, N groups of males = 33). Note that setting up experimental groups of unrelated individuals does not prevent the expression of social behaviours in *F. auricularia* adults (Weiß *et al.*, 2014) and that the use of unisexual groups of virgin individuals was done to prevent the potential effect of mating on the immune response (Lawniczak *et al.*, 2007). Five weeks later, we exposed each isolated individual and two individuals haphazardly sampled per experimental group to either a pathogen or a control solution (Fig. 1). To this end, each individual was dipped into a 2-mL Eppendorf tube previously filled with 500 μL of either a conidiospores solution of *M. brunneum* diluted in 0.05% Tween (10^7 spores mL^{-1}), or a control spore-free solution of 0.05% Tween. Every

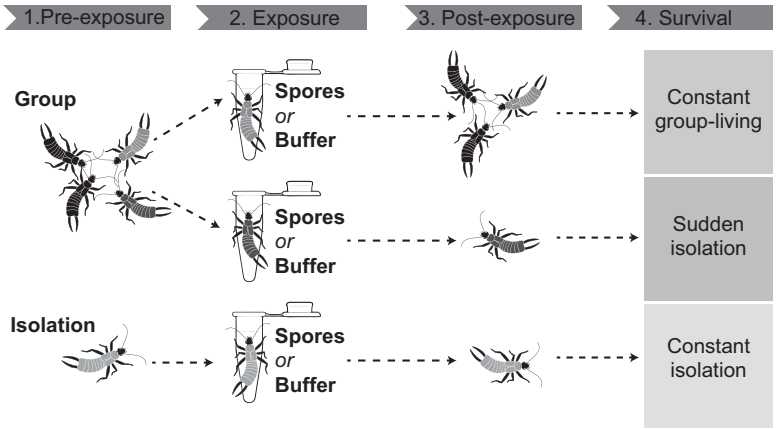

Fig. 1 Experimental design.

individual previously exposed to the pathogen or the control solution was then marked by clipping the tip of one of its two folded wings, and set up in a new Petri dish to obtain the three following treatments: individuals previously maintained in experimental groups were either returned to their original, nonexposed group members (constant group-living) or isolated immediately after exposure (sudden isolation), whereas individuals that were previously isolated were maintained in isolation after exposure (constant isolation)(Fig. 1). Each of these combinations included 17–21 replicates per sex and per type of exposure (see Fig. 2 for detailed numbers). The survival of every pathogen- and control-exposed individual was then recorded daily for the following 25 days. Note that two individuals per group were used to obtain the constant group-living and sudden isolation treatments. Petri dishes had a 10 cm diameter, were furnished with humid sand and provided with *ad libitum* standard food [food composition detailed in Kramer *et al.* (2015)]. *Metarhizium brunneum* was obtained from a strain isolated from soil samples in Switzerland and previously genotyped for identification (Reber & Chapuisat, 2011).

The effects of group-living on adult survival were tested using a Cox proportional hazard regression model allowing for censored data; that is, adults alive 25 days after exposure. Sex, exposure (spores or control), social treatment (constant group-living sudden

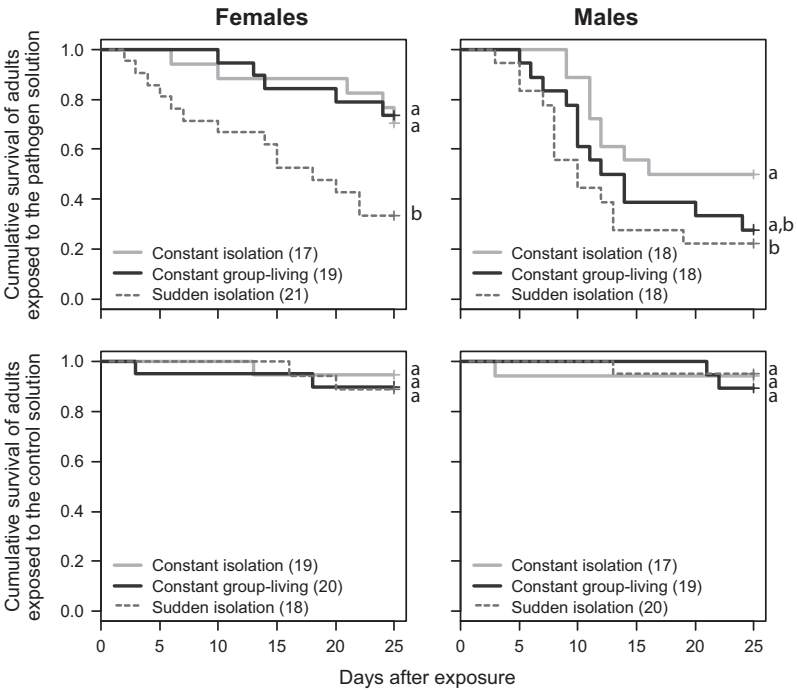

Fig. 2 Effects of pathogen exposure and social treatment on adults' survival rates. Sample sizes are given between brackets. The annotation with different letters indicates a *P*-value < 0.05.

isolation or constant isolation) and all interactions among these factors were entered as explanatory factors in this model. To control for the nonindependence of individuals used in the sudden isolation and constant group-living treatments, the ID of each experimental group (and constantly isolated individual) was entered into the Cox model using the *frailty* argument. Because a triple interaction between sex, exposure and social treatment determined the survival rates (Table S1, LR $\chi^2_2 = 46.90$, $P < 0.0001$), we split the data set per type of exposure (i.e. control and spore) and used the resulting subsets to conduct two Cox models (thereafter called control- and spore-subset Cox models) with sex, social treatment, their interaction and the *frailty* argument as explanatory variables. In the spore-subset Cox model, the significant interaction between sex and social treatment (see Results) was explored by comparing the effects of social treatments for each sex using two additional Cox models (called male- and female-subset Cox models), as well as by testing the effect of sex for each social treatment using log-rank tests. All statistical analyses were conducted using the software R v.3.2.1 (http://www.r-project.org/) loaded with the packages *car* and *survival*.

Results

Overall, 60 of the 111 (54.0%) infected and 9 of the 115 (7.8%) control adults died during the experiment (Fig. 2). Social treatments and sex interacted to shape the survival rate of infected adults (spore-subset Cox model; sex: LR $\chi^2_1 = 10.56$, $P = 0.001$; social treatment: LR $\chi^2_2 = 7.21$, $P = 0.027$; interaction: LR $\chi^2_2 = 45.20$, $P < 0.0001$), but had no effect on the survival of control adults (control-subset Cox model; sex: LR $\chi^2_1 = 0.12$, $P = 0.734$; social treatment: LR $\chi^2_2 = 0.27$, $P = 0.875$; interaction: LR $\chi^2_2 = 0.26$, $P = 0.880$). In infected females, the overall effect of social treatment (female-subset Cox model; LR $\chi^2_2 = 7.09$, $P = 0.029$) revealed that they survived less well after sudden isolation than when maintained in constant isolation (model coefficients $\pm$ SE = -1.23 $\pm$ 0.52, likelihood ratio (LR) $\chi^2_1 = 5.52$, $P = 0.019$) or in constant group-living (coefficients $\pm$ SE = -1.33 ± 0.52, LR $\chi^2_1 = 6.46$, $P = 0.011$), whereas constant isolation and constant group-living lead to similarly high survival rates (coefficients $\pm$ SE = 0.09 ± 0.63, LR $\chi^2_1 = 0.02$, $P = 0.880$). By contrast in infected males, the overall effect of social treatment (male-subset Cox model; LR $\chi^2_2 = 43.94$, $P < 0.0001$) reflected that infected males survived better in constant than in sudden isolation treatments (Fig. 2; coefficients $\pm$ SE = -1.55 ± 0.58, LR $\chi^2_1 = 7.16$, $P = 0.008$), but at comparable levels in constant isolation and constant group-living (Fig. 2; coefficients $\pm$ SE = -0.88 ± 0.57, LR $\chi^2_1 = 2.42$, $P = 0.120$) and in constant group-living and sudden isolation (coefficients $\pm$ SE = 0.68 ± 0.43, LR $\chi^2_1 = 2.52$, $P = 0.110$). Interestingly, infected males survived less well than infected females when maintained in the constant group-living treatment (Fig. 2; log-rank test; $\chi^2_1 = 9.5$, $P = 0.002$), whereas this sex difference was not found in the two other treatments (constant isolation: $\chi^2_1 = 1.8$, $P = 0.181$; sudden isolation: $\chi^2_1 = 1.2$, $P = 0.280$).

Discussion

In group-living species, the presence of conspecifics is often thought to be of key importance to ensure the survival of pathogen-exposed individuals, because it triggers a higher investment into personal immunity (i.e. DDP; Wilson & Cotter, 2009) and/or allows the expression of social immunity (Schmid-Hempel, 1998; Cremer *et al.*, 2007; Meunier, 2015). Here, we demonstrated that even if pathogen-exposed *F. auricularia* females survived better with group members than when suddenly isolated, this effect solely resulted from the transient cost of a sudden social isolation. Interestingly, our results also showed that in earwigs males, this cost of sudden social isolation only occured in comparison with constant isolation, but not compared to group-living. Finally, it is important to note that males and females that were not exposed to a pathogen exhibited only very low mortality during our experiment, and this mortality was independent of their social environment.

Our results reveal that living in a stable group may not only shape individuals' survival after pathogen exposure through the benefits of DDP or social immunity (as reported in many group-living insects; see Wilson & Reeson, 1998; Cremer *et al.*, 2007; Wilson & Cotter, 2009; Meunier, 2015), but also does it by preventing the immune-related cost of a sudden isolation. Interestingly, this cost was found in a species with a temporary and facultative form of group-living (Costa, 2006; Weiß *et al.*, 2014), suggesting that isolated individuals in species exhibiting permanent and obligatory social life, such as eusocial insects, are also (and maybe even more) likely to suffer from reduced resistance against pathogens. These findings thus call for further studies investigating the negative effects of social isolation on survival after pathogen exposure across social systems, as well as comparing the importance of these effects to the one of social immunity and DDP.

Social deprivation is a well-known source of stress across group-living species. For instance, it has been shown to increase the level of energetically costly activities in ants (Koto *et al.*, 2015), reduce willingness to interact socially in cockroaches (Lihoreau *et al.*, 2009), induce the expression of depressive behaviours in prairie voles (McNeal *et al.*, 2014), favour the development of metabolic diseases in mice and rabbits (Nonogaki *et al.*, 2007; Nation *et al.*, 2008), as well as to reduce lifespan in numerous species from different taxa (Boulay *et al.*, 1999; Lewis *et al.*, 2000; Ruan & Wu, 2008; Holt-Lunstad *et al.*, 2010; Modlmeier *et al.*, 2013; Koto *et al.*, 2015). Interestingly in female earwigs, we found that the cost of

sudden social deprivation only affected the survival of pathogen-exposed individuals, and that it specifically targeted their capability to fight against an infection. Although this study did not measure physiological parameters, our results suggest that sudden social isolation altered one or multiple mediators of the personal immune system of earwig females (e.g. Rantala *et al.*, 2007), such as haemocyte number and phenoloxidase and antimicrobial activities (Beckage, 2008). Indeed, the activity/concentration of these mediators is known to be stress sensitive in several insect species (Adamo, 2012). Moreover, the stress induced by social isolation is known to temporarily impair the concentration/activity of these mediators in vertebrates (e.g. Hawkley & Cacioppo, 2003; Hawkley *et al.*, 2015), even if these effects are only poorly understood in invertebrates (see Miller & Simpson, 2010).

Our results also suggest that living in a group of males just before pathogen exposure entails a cost in terms of resistance against infection, but that this cost is less apparent – or even absent – if males continue to live in a group after pathogen exposure. Specifically, males isolated after pathogen exposure survived less well if they were previously maintained in groups (sudden isolation) as compared to isolation (constant isolation), whereas they survived as well in groups (constant group-living) as in isolation (sudden and constant isolation) after pathogen exposure. These results, together with the one showing that infected males survived less well than females in the constant group-living treatment, shed light on a male-specific cost of group-living in terms of resistance against pathogens. This cost is likely to reflect the aggressiveness typically expressed among earwig males (Forslund, 2000; Weiß *et al.*, 2014), as high levels of aggressiveness are often known to trade off with immunocompetence (as shown in other insect species; e.g. Contreras-Garduño *et al.*, 2009; Adamo *et al.*, 2015). Conversely, the absence of an effect of group-living on survival after pathogen exposure may simply result from the lower activity of the infected males and/or their limited social interactions (e.g. infected workers in ants, Bos *et al.*, 2012). The effects of intrasexual interactions on the immunocompetence of earwig males, as well as the role of infection in their social behaviours, will be investigated in further studies.

To conclude, this study demonstrates that in a group-living species, the stress of a sudden isolation dramatically reduces individual's capability to fight against pathogen infection. This result gives a new perspective on the role of pathogen pressure in social evolution, as it shows that an apparently nonadaptive, personal immune process (i.e. a stress of social isolation) may help to maintain group-living under pathogenic environments, just as social immunity and DDP (Cremer *et al.*, 2007; Cotter & Kilner, 2010a; Meunier, 2015). Importantly, the immune-related costs of social isolation were only transient, suggesting that they may no longer enforce group-living once individuals experience isolation for some time. This recovery process could be crucial in female earwigs, as they typically become solitary prior to and during the period of egg care to limit the risk of egg cannibalism by conspecifics (Miller *et al.*, 2011). More generally, our results suggest that the addition of behavioural and/or physiological measurements (as well as a constant isolation treatment) is of key importance to properly interpret survival differences between grouped and isolated individuals as a support for social immunity or more generally, as a benefit of social interactions against pathogen infection. Overall, our findings stress that the maintenance of social life may not only rely on the fitness benefits entailed by the presence of group members, but also on the costs entailed by their sudden – yet not prolonged – absence.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article: **Table S1** Interaction between exposure, social treatment and sex on survival after pathogen exposure.

Data deposited at Dryad: doi: 10.5061/dryad.h5f5n

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