Division of labour in the black garden ant (Lasius niger) leads to three distinct proteomes

Martin Quque, Margaux Benhaim-Delarbre, Jean-Louis Deneubourg, Cédric Sueur, François Criscuolo, Fabrice Bertile

Division of labour in the black garden ant (*Lasius niger*) leads to three distinct proteomes

Martin Quque*[1], Margaux Benhaim-Delarbre[1], Jean-Louis Deneubourg[2], Cédric Sueur[1], François Criscuolo[1ǂ], Fabrice Bertile[1ǂ]

[1] Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France – 23 rue du Loess, 67037 Strasbourg Cedex 2, France
[2] Université Libre de Bruxelles, CPBT, CP231 Av. F. Roosevelt 50 1050 Bruxelles, Belgique
ǂ Share co-seniorship of the paper
* Correspondence: martin.quque@iphc.cnrs.fr - +33 388 106 951

Astract: Task specialization in social insects leads to striking intra-specific differences in behaviour, morphology, physiology and longevity, but the underlying mechanisms remain not yet fully understood. Adult colonies of black garden ants (*Lasius niger*) have a single queen fertilized by one or a small number of males. The inter-individual genetic variability is thus relatively low, making it easier to focus on the individual molecular differences linked to the division of labour. Mass spectrometry-based proteomics enabled us to highlight which biological functions create the difference between queens, foragers and nest-workers. The proteome of each caste reflected nicely their social role: e.g., reproduction for queens, pesticide resistance for foragers – that are the most exposed to environmental risk factors – and, interestingly, digestion for nest-workers, thus highlighting proteomic profiles differences even among workers. Furthermore, our exploratory approach suggests energy trade-off mechanisms – in connection with the theory of social immunity – that might explain the difference in longevity between queens and workers. This study brings evidence that proteomics is able to highlight the subtle mechanisms of molecular regulation induced by social organization.

Keywords: task specialization, social insect, trade-off, social immunity, longevity, mass spectrometry

1. Introduction

Animal species display different schemes of social organization – from solitary to eusocial species. Eusociality exists in certain mammals (Burda, Honeycutt, Begall, Locker-Grütjen, & Scharff, 2000), crustaceans (Duffy, Morrison, & R;íos, 2000) and insects (Wilson, 1971). The latter include eusocial Hymenoptera (wasps, bees, ants) and termites, the complex social organization of which is based on division of labour. Each individual belongs to a caste and displays a caste-specific set of behaviours. While the queen's main role is to produce offspring, the task specialization among workers is highly species-dependent and can result in a broad range of sizes and shapes within the same species (Harvell, 1994; Jeanne, 1986; Morton Wheeler, 1908; Seeley, 1986). Castes also differ in terms of longevity, queens reaching a dramatically longer lifespan than workers, living up to ten-fold longer (Keller & Genoud, 1997). The reproductive division of labour leads to a higher concentration of ecdysteroids and vitellogenin in reproductive individuals (Gospocic et al., 2017; Robinson, Strambi, Strambi, & Feldlaufer, 1991). Intriguingly, hormone concentrations do not reflect only the reproductive status but also the task specialization among workers, particularly the trio constituted by Insulin/Insulin-like growth factor Signalling, vitellogenin and juvenile hormone (Azevedo, Zanuncio, Delabie, & Serrão, 2011; Corona et al., 2013; Guidugli et al., 2005; Kohlmeier, Feldmeyer, & Foitzik, 2018; Libbrecht et al., 2013; Nelson, Ihle, Fondrk, Page Jr, & Amdam, 2007). Thus, resulting from division of labour in eusocial insects, genetically close individuals may nevertheless greatly differ from each other in terms of behaviour, morphology, physiology and longevity.

Genomics and proteomics picture different levels of gene expression (Gygi, Rochon, Franza, & Aebersold, 1999; Hunt et al., 2010). Studying differences between individuals at the proteome level allows consideration of changes which are likely closer to phenotypic variation than, for instance, gene expression (Baer & Millar, 2016). The study of the molecular basis of social life by genomic tools (*i.e.* sociogenomics) has already led to the identification of numerous genes (Robinson, Grozinger, & Whitfield, 2005; Sumner, 2006). By contrast, proteomics studies in social insects are less focused on behaviour and social interaction, concern mainly the honeybee biology (but (LeBoeuf et al., n.d.)), and are biased toward larvae rather than adults. In the latter case, the proteome of queen-destined and worker-destined larvae has been shown to differ in protein quantity for the following processes: resistance to oxidative stress, energy production, lipid metabolism, amino acid metabolism, development, protein biosynthesis, protein folding and cytoskeleton (Li et al., 2010). Larval mitochondrial (Begna, Fang, Feng, & Li, 2011) and larval nuclear (Begna, Han, Feng, Fang, & Li, 2012) proteome studies have shown similar results, as well as the comparison at an adult stage of antennal proteome between drones (male bees), workers and queens (Fang et al., 2012).

Black garden ants (*Lasius niger*) combine low genetic variations and a marked division of labour. Even though worker's ovaries are functional (Have, Boomsma, & Menken, 1988), the queen monopolizes the reproduction, she is larger and lives far longer than workers (Parker, 2010). These elements appoint black garden ants as a wise choice to stimulate the field of "socioproteomics", *i.e.* to determine the influence of the social role on individual's proteome, and then phenotype. In ants, proteomics has been hitherto used to specifically analyse spermatheca or venom compositions (Malta et al., 2014; Wiese et al., 2006), and protein response to desertic conditions (Willot, Gueydan, & Aron, 2017). With the hypothesis that abundance of proteins specific to given tasks and/or related to longevity and reproduction physiology should differ between the castes, we compared the proteome of black garden ant individuals, with the aim to characterize the differences between queens, foraging workers and non-foraging workers (respectively referred to as foragers and nest-workers).

2. Materials and Methods

2.1. Animal model

The black garden ant (*Lasius niger*, Linnaeus 1758) is a very common species in Western Europe, in urban and rural areas (Sterry, 1997). They are omnivorous and widely known to farm aphids for the honeydew they excrete (Domisthorpe, 1927). In adult colonies, only one queen lays eggs (monogynous species). She is fertilized by one or two males, and very rarely by more (Boomsma & Have, 2002; Fjerdingstad, Gertsch, & Keller, 2002; Fjerdingstad, Keller, & Tregenza, 2004). The queen is 7-9 mm long and has an average lifespan of 20 years, whereas workers are 2.5-5 mm long and live for 3 years on average (Hölldobler & Wilson, 1990). Unlike in other species, black garden ant foragers and nest-workers do not morphologically differ from each other.

The colonies used in this study came from wild newly-mated queens captured on the site "Campus Plaine" at the Université Libre de Bruxelles (50°49'08.4"N 4°23'57.0"E) and tended during two years in lab. We removed the eggs to control that all individuals were 2 years old. Colonies, consisting of only females (queen and worker ants), were housed in IPHC-DEPE (Strasbourg, France) at a temperature of about 25°C with 50-60% relative humidity and were fed with sugar water (0.3M) and mealworms once a week. Even though no law regulates the care and use of insects, we applied internal animal welfare policies by minimizing the number of ants used in experiments and by preventing any form of avoidable suffering.

2.2. Caste identification

The worker castes differ from each other by their interaction pattern and spatial segregation (Mersch, Crespi, & Keller, 2013). Active individuals, spending time in the foraging area were identified as foragers. On the other hand, the nest-workers were identified by no move outside the nest and their tendency to form immobile clusters. To stimulate the foraging behaviour, we used a 4-day fast and then placed a high concentration sugar solution (1M) in a plastic tray. To ensure an optimal recruitment, we waited for 5 minutes after the fifth individual came to the food source, then picked up all the forager individuals seen at the food source for one hour. No foraging behaviour was noticed after this period in preliminary tests. We then collected the nest-workers. Ants were anesthetized by cold (0.5-1 min, -20°C). A pen filled with acrylic ink (Posca®) was used to mark their abdomen, with a different colour according to the caste. When they woke up, the ants were carefully watched for a few minutes. None of them exhibited any sign of aftereffects. They were then put back into their colony where usual food and water were provided. This process was repeated three times, every 48 hours, to reduce the number of false positive (starved nest-workers exiting from the nest) and false negative (non-recruited or non-captured forager).

2.3. Proteomic analysis

2.3.1. Sample preparation

We used 15 colonies, individuals of which were homogeneously distributed among the samples. One sample is made of three queens or ten workers (nest-workers or foragers). We had five samples per caste, except in nest-workers, where only four samples had a sufficiently high protein content to be analysed by mass spectrometry. Frozen ants were ground under liquid nitrogen for 45 seconds at 30Hz using a Mixer Mill MM400 (Retsch, Eragny Sur Oise, France), and total proteins were extracted from the resulting powder using 200µl of extraction buffer (8 M urea, 2 M thiourea, 0.1 M Ammonium Bicarbonate, 1% DTT, protease inhibitors; Sigma-Aldrich, Lyon, France). After sonication on ice (2 x 10 seconds, 135 watts) and centrifugation (2000 x g, 2 minutes) to eliminate cuticle residues, 8 volumes of cold acetone were added to samples that were kept at -20°C overnight. Precipitated proteins were pelleted by centrifugation (13500 x g, 20 minutes, 4°C), and after discarding supernatants, dissolved in Laemmli buffer (10 mM Tris pH 6.8, 1 mM EDTA, 5% β-mercaptoethanol, 5% SDS, 10% glycerol). Samples were centrifuged to eliminate the remaining cuticles (2,000 g, 2 minutes). Total protein concentrations were determined using the RC-DC Protein Assay kit (Bio-Rad, Hercules, CA, USA). At this stage, a reference sample comprising equal amounts

of all protein extracts was made, to be injected regularly during the whole experiment and thus allow QC-related measurements.

20 µg of proteins from each sample were electrophoresed on SDS-PAGE gels (12% polyacrylamide) for 60 minutes at 50 V followed by 15 minutes at 100 V. After protein fixation (50% ethanol, 3% phosphoric acid), gels were stained overnight using colloidal Coomassie Blue. For each lane, five 2mm bands were excised, and proteins were in-gel digested with trypsin (Promega, Madison, WI, USA; 120 ng/band) at 37°C overnight after de-staining, reduction (10mM DTT), alkylation (55mM iodoacetamide), and dehydration using a MassPrep station (Micromass, Waters, Milford, MA, USA). Tryptic peptides were extracted using 60% acetonitrile, 0.1% Formic acid in water for one hour at 450 rpm on an orbital shaker. The organic solvent was then eliminated using a vacuum centrifuge (SpeedVac, Savant, Thermoscientific, Waltham, MA, USA), and peptides were re-suspended in 90 µl of 1% acetonitrile, 0.1% formic acid in water. A set of reference peptides (iRT kit; Biognosys AG, Schlieren, Switzerland) was finally added to each sample prior to LC-MS/MS analyses.

2.3.2. Nano LC-MS/MS analyses

Samples were analysed on a nanoUPLC-system (nanoAcquity, Waters) coupled to a quadrupole-Orbitrap hybrid mass spectrometer (Q-Exactive Plus, Thermo Scientific, San Jose, CA, USA) using a randomized sequence within block injections. Each block consisted of one biological sample of each group plus the reference sample. To reduce carry-over, two solvent blank injections were included in between each sample. Briefly, one µl of each sample was concentrated/desalted on a Symmetry C18 pre-column (0.18 x 20 mm, 5 µm particle size; Waters) using a mobile phase composed of 99% of solvent A (0.1% formic acid in water) and 1% of solvent B (0.1% formic acid in acetonitrile) at a flow rate of 5 µl/min for 3 minutes. Afterwards, peptides were eluted using a UPLC separation column (BEH130 C18, 200 mm x 75 µm, 1.7 µm particle size; Waters) maintained at 60 °C with the following gradient: from 1% to 6% B in 30 seconds, from 6% to 35% B in 59.5 minutes.

Q-Exactive Plus was operated in positive ion mode with source temperature set to 250°C and spray voltage to 2.0 kV. Spectra were acquired through automatic switching between full MS and MS/MS scans. Full scan MS spectra (300-1800 m/z) were acquired at a resolution of 70,000 at m/z 200 with an automatic gain control (AGC) value set to 3×10^6 ions, a maximum injection time set to 50 ms, and the lock-mass option being enabled (polysiloxane, 445.12002 m/z). Up to 10 of the most intense precursors (with a minimum of 2 charges) per full MS scan were isolated using a 2 m/z window and fragmented using higher energy collisional dissociation (HCD), with normalised collision energy set to 27 eV and dynamic exclusion of already fragmented precursors set to 60 seconds. MS/MS spectra were acquired at a resolution of 17,500 at m/z 200 with an AGC value set to 1×10^5 and a maximum injection time set to 100 ms, and the peptide match selection option was turned on. The system was fully controlled by Xcalibur software (v3.0.63; Thermo Fisher Scientific). Peak intensities and retention times of reference peptides were monitored in a daily fashion.

2.3.3 Protein identification and quantification

MS raw data processing was performed using MaxQuant (v 1.5.3.30). Peak lists were searched against a UniProtKB-derived protein database created using the MSDA software suite (Carapito et al., 2014). The database contained *Lasius niger* (TaxID 67767) protein sequences (February 2017; 18075 sequences) to which sequences of common contaminants were added (247 entries; contaminants.fasta included in MaxQuant). A minimal number of one peptide (unique or razor) was required for protein identification. A maximum number of one missed cleavage and a false discovery rate (FDR) of 1% for both peptide-spectrum matches (minimum length of seven amino acids) and proteins was accepted during identification. From the use of a *Lasius niger* protein database, we identified 57 "uncharacterized" proteins (~4% of all identified proteins) for which we searched known homologous proteins in the Protostomia clade. This was done by using BLAST searches (FASTA program v36; downloaded from http://fasta.bioch.virginia.edu/fasta_www2/fasta_down.shtml), and only the best hits were retained.

To validate this procedure, we automatically extracted orthology annotations and sequence domains of *Lasisus niger* uncharacterized proteins and of their homologues from the OrthoDB (Kriventseva et al., 2019) and InterPRO (Mitchell et al., 2019) resources. The relevance of the match between *Lasisus niger* uncharacterized proteins and their homologues was then checked manually.

Regarding quantification, data normalisation and protein abundance estimation were performed using the MaxLFQ (label-free quantification) option implemented in MaxQuant (Cox et al., 2014) using a "minimal ratio count" of one. "Match between runs" was enabled using a one minute time window after retention time alignment. Only unmodified peptides were considered for quantification (except those for which a modified counterpart was detected) while shared peptides were excluded. All other MaxQuant parameters were set as default.). Only proteins quantified with at least two unique peptides and detected in at least three samples in a given caste were kept for further analysis. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Vizcaíno et al., 2016) partner repository with the dataset identifier PXD006779.

Regarding quality controls, we found that the median coefficient of variation (CV) of retention times and raw intensity of iRT peptides when considering all injections was 0.96% and 22%, respectively. The median CV regarding the raw intensity of all quantified proteins across a repeated analysis of the reference sample was 16%. These different values support the good stability of the nanoLC-MS/MS system during the whole duration of analyses, and good reproducibility of protein abundance determination.

2.4. Protein selection procedure and PCA

In total, 2707 proteins were identified, of which 1325 fulfilled the criteria for accurate quantification (see above). This original dataset is available online in supplementary material (Table S1-S3). To properly run the PCA (principal component analysis); missing data were inferred by regularized iterative PCA algorithm (missMDA package; (Josse & Husson, 2016). The PCA was performed (FactoMineR, v.1.34; Lê et al., 2008) on all three castes, and also only the two workers castes separate from the queen caste, in order to have a more precise insight into potential differences. First, we used PCA as a filter to shrink the number of variables and only retain proteins giving rise to differences between the castes. Only 174 proteins for the three castes and 108 for the workers were strongly correlated to the axes ($p<0.05$) and faithfully projected ($cos^2 > 0.8$; Fig. S1 C and D). Most of the time, protein databases in insects are unfortunately patchy and based on predicted annotations. This makes an automatic annotation highly prone to misassignment. The protein functions were therefore "manually" attributed by using several database resources (Ortho DB, InterPro, UniProt) that contain annotations for proteins from the Protostomia clade that are homologous to *Lasius niger* proteins, as well as results from previous studies in insects (social insects whenever possible) to avoid misleading functional annotations. In the case of pleiotropic proteins, we kept all the different functions (proteins indicated in blue in Table S2-S3) and did not arbitrarily choose one among the others. Finally, 255 proteins were kept for further analyses (**Fig. 1**), and they were clustered according to their functions (categories in **Table 1**). These functional groups were used as variables to build the axes of the second set of PCAs (**Fig. 2**), in order to determine which biological functions allow discriminating queens from nest-workers from foragers."

Most of the time, proteomics databases in insects are unfortunately patchy and based on predicted annotations. This makes an automatic annotation highly prone to misassignment. The protein functions were therefore "manually" attributed by crossing several databases (Ortho DB, InterPro, UniProt) and actual studies in insects (social insects whenever possible) to avoid misleading functional annotations. In the case of pleiotropic proteins, we kept all the different functions (proteins indicated in blue in Table S2-S3) and did not arbitrarily choose one among others. Finally, 255 proteins were kept for further analyses (**Fig. 1**), and they were clustered according to their functions (categories in **Table 1**). These functional groups were used as variables

to build the axes of the second set of PCAs (**Fig. 2**), in order to determine which biological functions allow discriminating queens from nest-workers from foragers.

2.5. Statistical tests

Statistical tests were performed using R software (R Core Team, v3.4, 2017) at the significance threshold $\alpha = 5\%$. To statistically test whether any biological function isolates one caste from another, we used the PCA's coordinates as variables. With them, we performed Kruskal-Wallis (KW) tests (>2 modalities, heteroscedasticity) and Wilcoxon rank sum test (2 modalities, homoscedasticity). If the KW tests were significant, they were followed by Conover-Iman posthoc test with Bonferroni correction (conover.test v.1.1.5; Alexis Dinno). Homoscedasticity was assessed by Bartlett test.

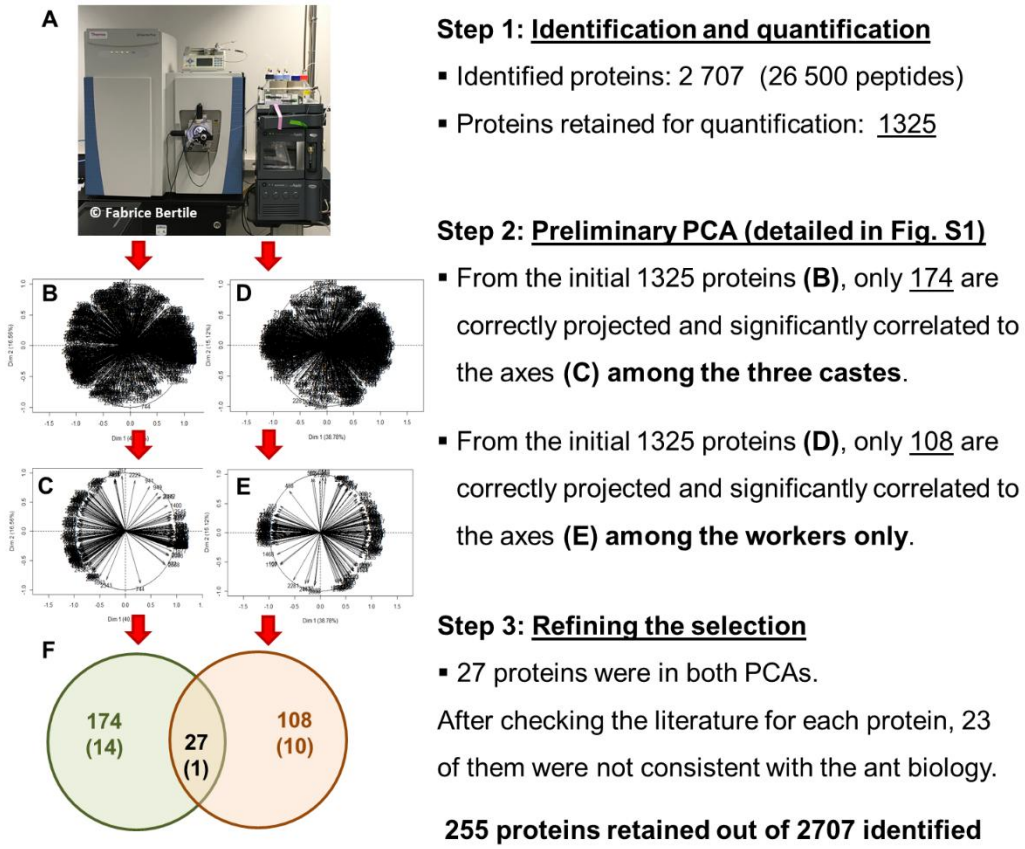

Fig. 1. Protein selection procedure. The underlined numbers are the subset in the step n used in the step n+1. **A:** First, the mass spectrometer and the MaxQuant Software were used to analyse the proteins. Amongst the 2707 identified proteins, 1325 could be quantified. One PCA regarding the three castes **(B)** and one regarding the workers only **(D)** were performed with these proteins. Only the proteins properly projected (cos^2>0,8) and significantly correlated to the axes (p<0,05) were kept for the three castes **(C)** and the workers only **(E)**. **F:** The number in brackets refers to the number of inconsistent proteins among the total number. A total of 23 proteins did not show strong evidence to be involved in insect's processes or in evolutionarily conserved mechanisms. 27 proteins were redundant between the two PCAs.The 255 proteins remaining at the end of this procedure were clustered according to their biological function in the further analysis. *Photo credits: Fabrice Bertile, co-author of the publication.*

Table 1. Functional groups retained to build the axes of the PCA. Functional category's names are adapted from GO term annotations (www.geneontology.org) of proteins homologous to *Lasius niger* proteins, identified using several database resources (Ortho DB, InterPro, UniProt), as well as literature examination. The IDs corresponds to the numbers found on the PCA plots (figures 2 and S1). The attribution of proteins to functional groups is detailed in the 'Materials and Methods' section.

Functional group	ID	Description
Cell Activity	1	Protein involved in several mechanisms highlighting general cell activity: transcription/translation, ATP synthesis…
Ageing +	2a	Direct and/or strong association with the individual ageing status.
Ageing -	2b	Direct and/or strong association with a slower ageing rate or extended lifespan.
Apoptosis +	3a	Inducing or fostering apoptosis.
Apoptosis -	3b	Inhibiting or delaying apoptosis.
Tissue Growth	4	Tissue growth especially during embryonic development.
Chaperone	5	Protecting the cell against harmful conditions (oxidative stress, pH variation…). Ensuring a proper protein folding.
Cell Cycle	6	Controlling cell cycle (mitosis/meiosis, blocking cell cycle…).
Cytoskeleton	7	Part of the cytoskeleton or associated with (actin, dynein, kinesin…).
Detoxification	8	Soma repair after a stressful event.
Digestion	9	Protein involved in the digestion metabolism.
Cell Dynamics	10	Non-focused action proteins involved in structural cell mechanisms: controlling cell shape, adhesion…
GnExpression+	11a	Activating gene expression (transcription and/or translation).
GnExpression-	11b	Inhibiting gene expression (transcription and/or translation).
Calcium Homeostasis	12	Regulating the calcium level.
Human Pathologies	13	Human diseases – degenerative most of the time (*e.g.* Alzheimer).
Xenobiotics Detox	14	Resistance to chemicals, especially pesticides.
Immunity	15	Resistance to pathogens.
Larvae	16	Proteins related to larval development.
NclAcid Metabolism	17	Nucleic acids synthesis or modification.
Energy Metabolism	18	Protein involved at least in one of the following pathways: glycolysis, Krebs cycle, gluconeogenesis, ATP synthesis.
Glucid Metabolism	19	Glucid modification, not for direct use in glycolysis or Krebs cycle
Lipid Metabolism	20	Lipid modification, not in an energetic purpose.
Protein Metabolism	21	Protein synthesis or modification.
Muscles	22	Protein required for muscle contraction.
IR	23	Irrelevant: unknown function or inconsistent in *L. niger*
Cell Proliferation	24	Protein directly involved in cell proliferation.
Glucid Recycling	25	Breakdown of glucids to provide the cell with new fatty materials.
Protein Recycling	26	Breakdown of proteins to provide the cell with new amino acids.
Redox	27	Promoting redox reactions in physiological conditions, ensuring the redox balance within the cell.
Reproduction	28	Proteins related to the gametes.
Secretion	29	Secreted proteins: hormones, pheromones, in saliva.
Nervous System (NS)	30	Growth, maintenance and repair of the nervous system.
Sensitive NS	31	Protein involved in the sensitive nervous system.
Membrane Trafficking	32	Protein involved in membrane trafficking between RE and Golgi or

related to other transport vesicles (synapses, endo/exocytosis).

3. Results

3.1. Biological functions splitting the castes according to the PCAs

In the supplementary material section, we supply accession numbers, names and relative amounts of the 1325 quantified proteins (Table S1 B). Table S2 and S3 aggregate the 255 retained proteins and their functional category. We identified 35 functional groups (**Table 1**) according to our selection procedure.

Regarding the three-caste analyses, the first two dimensions (**Fig. 2 A**) of the PCA explained 82.41% of the variance, which is considered significant (Table S4). Only the first axis statistically separated all castes from each other (χ^2KW_{axe1} = 9.926, p_{axe1} = 0.01 and χ^2KW_{axe2} =0.591, p_{axe2} = 0.744): forager-nest-worker (t=1.82, p=0.048), queen-nest-worker (t= -3.71, p=0.002), forager-queen (t=-5.86, p<0.001). Queens were mainly characterised by proteins involved in somatic maintenance – mechanisms aiming to avoid or repair damages to macromolecules – cell division, gene expression regulation and trafficking. While workers expressed more proteins related to metabolic pathways (except lipid metabolism) and sensitive nervous system or immunity.

In the second PCA (workers only), the first two dimensions (**Fig. 2 B**) explained 87.94% of the variance, which is considered significant (Table S4). The second axis isolated nest-workers from foragers, but the first axis did not (W_{axe1} = 16, p_{axe1} = 0.191 and W_{axe2} = 20, p_{axe2} = 0.016). Foragers presented more proteins associated with xenobiotic detoxification mechanisms, whereas nest-workers had more proteins involved in digestion metabolism.

3.2. Proteins related to the ToR pathway

The Target of Rapamycin (ToR) protein belongs to the serine/threonine kinase family (Helliwell et al., 1994). A growing literature shows the implication of the ToR pathway in ageing-related diseases (Skike & Galvan, 2018) and its evolutionarily conserved ability to shorten lifespan in various taxa (Powers, Kaeberlein, Caldwell, Kennedy, & Fields, 2006). We were thus interested in knowing whether the amount of ToR-related proteins would differ between the castes. Four proteins involved in the ToR pathway were significantly different between castes: striatin-3 isoform x2 (χ^2_{KW} = 10.73, p < 0.001), peptidyl-prolyl cis-trans isomerase (χ^2_{KW} = 9.23, p = 0.01), ubiquilin-1-like isoform 2 protein (χ^2_{KW} = 6.17, p = 0.04) and eukaryotic translation initiation factor 4e (χ^2_{KW} = 6.94, p = 0.03). Only the cAMP-dependent protein kinase catalytic subunit was not significant (χ^2_{KW} = 4.06, p = 0.13). However, they are only marginally related to the ToR pathway and could be involved in other signalling pathways. Hence, we did not perform further analyses.

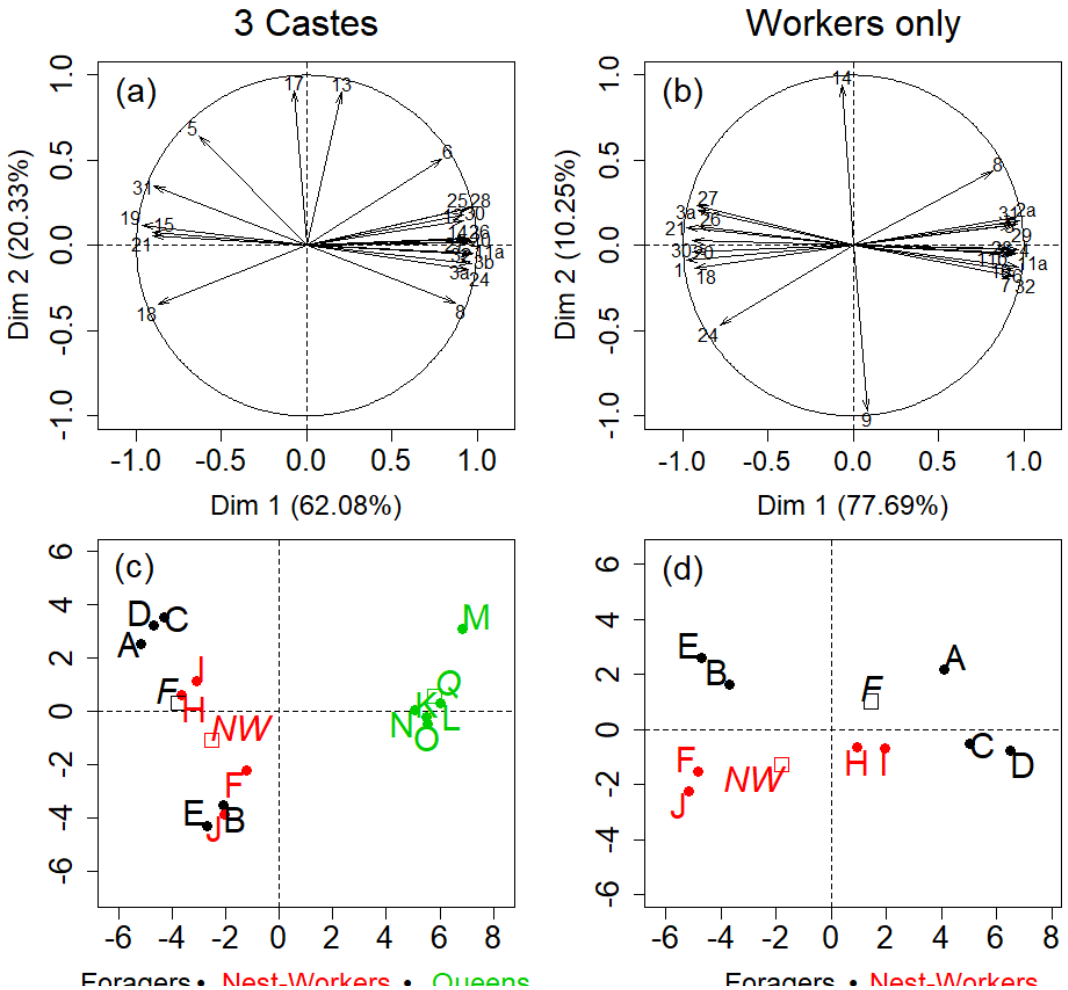

Fig. 2 PCA amongst the castes of *L. niger*. Left: charts regarding the three castes. 14 ant pools and 33 variables (biological functions). / **Right:** charts regarding the workers only. 9 ant pools and 25 variables (biological functions). **(a) and (b)**: functional groups correlating with at least one of the axes ($p<0.05$) and with a $\cos^2 > 0.8$, identified by a number (names and functions in **Table 1**). **(c) and (d):** representation of the individuals. Similar coordinates mean the individuals show a similar level of protein expression in the same biological functions. The empty squares with a capital letter (Q=queens, NW=nest-workers, F=foragers) indicate the average coordinates for each caste.

4. Discussion

Our proteomic analysis combined with a PCA-based protein selection highlighted biological functions specific to each caste. The exhaustive list of selected proteins and related functions is available in supplementary data (Table S2 and S3). Below, we focus the discussion on some functions (Table 1) or life-history traits that were either different between queens and workers, or, within the workers, between foragers and nest-workers. First, we focus on identified caste-specific functions that define the worker castes. Second, we propose other sources of individual variation amongst the workers. Third, based on observed differences in queens and workers profiles, we discuss possible mechanisms involved in the large difference in longevity. This leads us to question the usual fecundity/lifespan trade-off by considering the energetic cost of an active immune system in a so particular social context.

4.1. Division of labour has multiple proteomic consequences in workers

4.1.1. Sensory system

We found a homologue protein (apd-3-like protein) to the bee's apd3 protein which is, according to a genomic study, related to the olfactive/gustative function in the antennae (Antony et

al., 2016). SAP47 was also part of this functional group and is involved in the learning process of smells and pictures in Drosophila's larvae (Saumweber et al., 2011). Ant communication is (almost) all about pheromones. Nest building, foraging, social identification: all rely on those signals (Beckers, Deneubourg, & Goss, 1993; Khuong et al., 2016; Yan et al., 2017). Moreover, worker ants must also decipher the environmental cues. For instance, foragers must find the appropriate food sources according to colony needs within an environment full of non-specific odorants. Hence, it is not surprising to see in workers high levels of proteins related to the sensitive nervous system (**Fig 2 C**), allowing them to detect and analyse those specific and non-specific olfactory cues. This hypothesis is supported by a genomics study in *L. niger*, where workers up-regulate the Ln385_5 gene, involved in odorant binding (Graeff, Jemielity, Parker, Parker, & Keller, 2007).

4.1.2. Immunity

Workers had on average a higher amount of proteins associated with the immune system (*e.g.* arginine kinase, T-cell immunomodulatory protein). Some studies have opposing results regarding the expression of immunity-related genes in ants (Graeff et al., 2007) or bees (Grozinger, Fan, Hoover, & Winston, 2007). Moreover, ferritin, known to withhold iron from invading pathogens (Ong, Wang, Zhu, Ho, & Ding, 2005) has only been found in queens in our analysis. On the other hand, a study in *Melipona quadrifasciata* (C. C. Judice et al., 2006) has found an up-regulation in workers of a gene coding for a scavenger receptor involved in the immune response. The relationship between the caste and the immune system seems hence to be equivocal.

At first glance, the fact that queens are more susceptible to pathogens because of weak immune defences does not sound evolutionary stable. High productivity in laying eggs is pointless if ant queens do not survive the first encountered pathogen. Moreover, we know that group living makes individuals more prone to infection (Godfrey, Bull, Murray, & Gardner, 2006; Schmid-Hempel, 1998). To overcome these issues, eusocial insects have evolved a combination of behavioural responses, called social immunity (details in Cremer et al., 2007). For instance, infected individuals are less involved in interactions and they can sometimes even be killed by their own colony. Cremer et al. also suggest that the structure of the interaction network might be shaped in a prophylactic way to prevent pathogens from reaching the queen. The queen would be thus "socially" protected from pathogens. In this context, a weaker immune system would not be an inevitably fatal issue. Supporting this assumption, a phylogenetic study in five insect species has shown that the larger the colony size is, the weaker the melanization response (López-Uribe, Sconiers, Frank, Dunn, & Tarpy, 2016). In addition, ants – similarly to other eusocial insect species – have fewer genes involved in immune functions than less social insects (Libbrecht et al., 2013), what brings evidence that proper social structure can allow for a reduced immune system.

4.1.3 Differences within the worker caste

Foragers differ from nest-workers by their higher amount of proteins involved in insecticide resistance – mostly cytochrome P450 (CYP). The role of CYP in insecticide detoxification is well documented (Oppert et al., 2015; Werck-Reichhart & Feyereisen, 2000). Because of their supplying role, foragers are directly exposed to pesticides. This larger amount of pesticide-degrading proteins may help them to face environmental toxic chemicals. Once detoxified by foragers, the food can be safely distributed to the whole colony. Overexpression of the CYP gene has also been found in the worker caste of *Melipona quadrifasciata* (C. Judice, Hartfelder, & Pereira, 2004). This could indicate a common response to insecticides among social insects. A similar response has been observed in honey bee, where the activity of two detoxification enzymes increases when workers begin to forage (Smirle & Winston, 2011). Yet, CYP is also involved in metabolic functions in insects such as hormone degradation (Feyereisen, 1999) and we cannot rule out at that stage other metabolic implications.

We found that a larger amount of digestive proteins (essentially alpha-amylase) are expressed in nest-workers. By storing food excess (Lenoir, 1979) and pre-digesting it, nest-workers can make it

quickly available for further use, thus enhancing the fitness of the whole colony by buffering environmental unpredictability in food resources, but also by increasing food processing by conspecifics. Nest-workers may also pre-digest the food for castes that do not perform this task very well. According to several studies, ant larvae do not require help to digest food (Cassill, Butler, Vinson, & Wheeler, 2005; Erthal, Peres Silva, & Ian Samuels, 2007; Went, Wheeler, & Wheeler, 1972). The pre-digested food could be more useful for the queen, allowing her synthesizing fewer digestive proteins and saving energy for other costly life history traits (*i.e.* reproduction). Whether this may be of any advantage for other adult castes must be tested by accurately measuring digestive proteins levels of expression in nest-workers and foragers.

4.2. Other sources of variation within the workers

Social castes appeared to be of major importance to explain the proteome variability among adult individuals in the black garden ant. Nevertheless, unexplained variance remained (**Fig. 2 C, D**) and nest-workers and foragers were not all perfectly collocated, suggesting other sources of individual variation.Tan et al. (2017) highlighted that diapause can affect the proteome of the cabbage beetle (*Colaphellus bowringi)*. All the colonies were nonetheless under the same day light and temperature conditions, it is hence unlikely that some individuals enter diapause and others did not.

Usually in ants, there is a temporal division of labour: workers specialize with their age (Jeanne, 1986). However, the colony needs (*e.g.* more brood to feed, galleries to dig) can induce individuals to change caste – regardless of the age (Robinson, 1992). For instance, if most of the foragers die from predation, some of the nest-worker workers become foragers to maintain food supply of the colony. They become foragers earlier than expected. Therefore, we might find individuals of different age within the same caste. Age-related phenotype including physiological traits would consequently not be homogenous and thus explain part of the individual variability within a caste. However, we expect this age-independent caste switching effect to be minimal, since ants were reared under constant laboratory conditions for more than two years. Furthermore, all the workers used in this study were at least two years old, damping the potential impact on the proteome of a big age difference between worker castes. Although the effect of age is mitigated, we cannot be 100% sure that it does not influence our results, at least in part. Under this assumption, the age effect can be either independent or confounded with the effect of the caste. If it is independent, then it could explain the remaining variation not attributable to the caste (axis 2 of the first PCA and axis 1 of the second one). If age and cate effects are confounded, then this remaining variation would be due to a third factor, which remains unknown so far.

4.3. A possibly multifactorial gap in lifespan

4.3.1. Energy metabolism

Proteins associated with energy metabolism were more abundant in worker ants. Most of the proteins forming this group are involved in the Krebs Cycle (*e.g.* NADH dehydrogenase, succinyl ligase, citrate synthase), ATP synthesis (ATP synthase) or lipid beta-oxidation (long-chain-fatty-acids ligase 3). This suggests a higher metabolic activity (potentially associated with higher metabolic rates) in workers than in queen ants. Oxygen consumption at the colony level was found to be higher in workers than in queens of fire ants (Vogt & Appel, 1999). Oxygen consumption was even higher in workers moving the most intensively (Ferral, Holloway, Li, Yin, & Hou, 2017) or the smaller ones (Calabi & Porter, 1989), raising the question whether metabolic rate is also an important determinant of lifespan in workers.

4.3.2. Somatic maintenance

Queen ants were, among others, characterized by higher amount of apoptosis-regulating proteins. As recently highlighted (Deursen, 2014), preventing the accumulation of senescent cells within tissues is a key determinant of an organism's lifespan and health. Killing dysfunctional cells seems also to be one of the keys to longevity (Berger et al., 2006; Ravikumar, Berger, Vacher, O'Kane,

& Rubinsztein, 2006; Tchkonia, Zhu, Deursen, Campisi, & Kirkland, 2013). The negative impact of senescent cells is mostly mediated through the Senescent Associated Secretory Pathway (Matjusaitis, Chin, Sarnoski, & Stolzing, 2016; Tchkonia et al., 2013). Contrarily, promoting senescence may also be beneficial through its implication in tissue repair (Jun & Lau, 2010) and tumour suppression (Collado, Blasco, & Serrano, 2007). Focusing on the dynamics of senescence markers over life in the different castes and in different species may be of interest in the near future to estimate how senescence control has co-evolved with both longevity and sociality in ants. Queens also had higher amount of proteins belonging to the two functional groups 'Detoxification' and 'Chaperone'. In these groups are found proteins involved in macromolecules restoration after stress (*e.g.* aldehyde dehydrogenase, selenium-binding protein 1-a), ensuring proper folding of proteins (GrpE protein homolog, T-complex protein 1) or regulating cell energy production during stress (mitochondrial UCP2). Higher protein quantities from these groups characterized the queens. A previous study in *L. niger* has also found that the expression of somatic maintenance genes is up-regulated in queens (Graeff et al., 2007). This suggests that the queen's longevity might, at least in part, result from a higher energy investment in preventing cell damages.

4.3.3. Reproduction and refinement of longevity trade-off in queens

As expected, the queen's reproductive role was confirmed by the analysis, since proteins related to reproduction were solely found in queen ants. The Reproduction functional group was only made of sperm-related proteins (e.g. sperm-associated antigen). This protein abundance can be explained by the spermathecal storage of sperm in queens. In our study and contrary to genomic studies (Graeff et al., 2007; Grozinger et al., 2007), vitellogenin was not overexpressed in the reproductive caste. As highlighted by Amdam et al. (2003), vitellogenin is also found in workers. The difference in protein quantity might not be sufficient to isolate workers from queens. During oogenesis, lipids are required for the biosynthesis of the egg cell membrane or lipoproteins (Engelmann, 1979), and a functional reproductive system synthesizes steroid hormones, which require lipid precursors (Hoffmann, 1980). Consistently, queens had on average a higher quantity of proteins involved in lipid transport (e.g. phospholipid-transporting ATPase, apolipoprotein D) or lipid synthesis (fatty acid synthase). Since energy is limited, investment in reproduction is done at the expense of other functions. Consequently, when a species or an individual is long-lived, we usually expect a lower energy investment in reproduction according to the fecundity/lifespan trade-off (Stearns, 1977). Queens of social insects do not seem to undergo this trade-off, as they are both long-lived and the only reproductive individual in the colony (Blacher, Huggins, & Bourke, 2017). This is notably the case in black garden ant queens characterized by intense reproduction combined with extreme lifespan – up to 28 years in *L. niger* (Parker, 2010). The solution could be not to consider a lifespan-vs-fecundity trade-off, but a lifespan-fecundity-vs-immunity trade-off. An active immune system is energy-consuming both at the individual (Moret & Schmid-Hempel, 2000) and colony level (Evans, Pettis, & Mueller, 2005). The investment in immunity has been shown to impair reproduction and/or growth (Kopp & Medzhitov, 2009). For instance, up-regulation of immune genes decreases reproductive success in urban blue tits (Capilla-Lasheras et al., 2017). Consequently, if the queen invests less in the immune system – as suggested by our data – she might save energy for reproduction or/and mechanisms aiming to avoid or repair damages to macromolecules.

4.3.4. ToR pathway in social insects

Proteins whose quantity differed between castes were downstream component and/or weakly related to the Tor pathway. We cannot therefore highlight a clear involvement of the ToR pathway in caste differentiation in black garden ants. Whereas, it is unequivocal in honey bee, where queen-destined larvae upregulate this signalling pathway relatively to worker-destined ones (Page & Amdam, 2007; Patel et al., 2007). As the queen-destined larvae are overfed, such a finding confirms the nutrient-sensitive role of ToR pathway to control growth according to the food availability. On the other hand, activation of the ToR pathway is strongly associated with a shorter lifespan among diverse taxa (Kapahi et al., 2010). The longevity secret of queen social insects might

be a ToR expression modulation depending on their age. When queens are still larvae, an active ToR pathway (stimulated by overfeeding) would allow somatic growth and ovaries maturation. Then, ToR expression would decrease with age, protecting the queens from senescence. The opposite scheme would take place in workers.

5. Conclusions

We showed that proteomics allows assessing fine molecular differences induced by task specialization in a social insect species. The non-targeted screen of the whole proteome highlighted a wide diversity of caste-dependent functions from immunity to reproduction to digestion to insecticide resistance. Our study also raises evolutionary questions about longevity and energy trade-offs in eusocial species, beyond the classical free-radical theory of ageing. Thanks to our exploratory approach, we now have a more global insight into all the functions that can be affected by the division of labour in a eusocial species. Some are well studied (*e.g.* social immunity), others less, especially in the adult stage (*e.g.* the ToR pathway, difference in the metabolism of digestion). We therefore hope to pave the way for future experiments to accurately test the numerous and diverse molecular mechanisms induced by a eusocial lifestyle.

Supplementary Materials: The following charts and tables are available online. Figure S1: PCAs used for variable selection (before clustering by biological function). Table S1: Raw data from the mass spectrometry-based proteome analysis, Table S2: Proteins used for the PCAs with the biological functions amongst the three castes, Table S3: Proteins used for the PCAs with the biological functions amongst workers only, Table S4: Inertia's 95th percentile for the first two dimensions of 10,000 PCAs. Tables S1-S3 present raw data and are available in a separated online repository (doi: 10.17632/xk4rpddxx6.1).

Author Contributions: Conceptualization, M.Q., C.S.,J-L.D., F.C., .FB.. ; methodology, FB, J-LD, MQ.; software, M.B-D., F.B., M.Q.; validation, F.B., M.B-D., M.Q.; formal analysis, M.Q.; resources, J-L.D., F.B., C.S., F.C.; data curation, F.B., M.B-D. ; writing—original draft preparation, M.Q., M.B-D., F.B.; writing—review and editing, C.S.,J-L.D., F.C., F.B.; visualization, M.Q.; supervision, C.S.,J-L.D., F.C., F.B.; project administration, C.S.,J-L.D., F.C., F.B.; funding acquisition, M.B-D, F.C., F.B., C.S.

Funding: This research was funded by the CNRS and Strasbourg University (H2E project; IdEx Unistra), the Proteomics French Infrastructure (ProFI; ANR-10-INSB-08-03). During the tenure of this study, M.B-D. was the recipient of a grant from the Strasbourg University (IdEx Unistra).

Acknowledgments: We thank Olivier Bles for providing ants and valuable advice all along the study. We also thank Dr Hélène Gachot-Neveu, Aurélie Kranitsky and Marie-Laure Rizzi for their precious work in the animal husbandry, and Dr Patrick Guterl for his help in bioinformatics analysis of proteomics data. We warmly thank Tracey Hammer for her careful proofreading of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

Antony, B., Soffan, A., Jakše, J., Abdelazim, M. M., Aldosari, S. A., Aldawood, A. S., & Pain, A. (2016). Identification of the genes involved in odorant reception and detection in the palm weevil Rhynchophorus ferrugineus , an important quarantine pest, by antennal transcriptome analysis. *BMC Genomics*, *17*(1), 69. https://doi.org/10.1186/s12864-016-2362-6

Azevedo, D. O., Zanuncio, J. C., Delabie, J. H. C., & Serrão, J. E. (2011). Temporal variation of vitellogenin synthesis in Ectatomma tuberculatum (Formicidae: Ectatomminae) workers. *Journal of Insect Physiology*, *57*(7), 972–977.

Baer, B., & Millar, A. H. (2016). Proteomics in evolutionary ecology. *Journal of Proteomics*, *135*, 4–11. https://doi.org/10.1016/j.jprot.2015.09.031

Beckers, R., Deneubourg, J.-L., & Goss, S. (1993). Modulation of trail laying in the ant Lasius niger (Hymenoptera: Formicidae) and its role in the collective selection of a food source. *Journal of Insect Behavior*, *6*(6), 751–759.

Begna, D., Fang, Y., Feng, M., & Li, J. (2011). Mitochondrial proteins differential expression during honeybee (Apis mellifera L.) queen and worker larvae caste determination. *Journal of Proteome Research*, *10*(9), 4263–4280.

Begna, D., Han, B., Feng, M., Fang, Y., & Li, J. (2012). Differential expressions of nuclear proteomes between honeybee (Apis mellifera L.) queen and worker larvae: A deep insight into caste pathway decisions. *Journal of Proteome Research*, *11*(2), 1317–1329.

Berger, Z., Ravikumar, B., Menzies, F. M., Oroz, L. G., Underwood, B. R., Pangalos, M. N., … Rubinsztein, D. C. (2006). Rapamycin alleviates toxicity of different aggregate-prone proteins. *Human Molecular Genetics*, *15*(3), 433–442. https://doi.org/10.1093/hmg/ddi458

Blacher, P., Huggins, T. J., & Bourke, A. F. G. (2017). Evolution of ageing, costs of reproduction and the fecundity–longevity trade-off in eusocial insects. *Proc. R. Soc. B*, *284*(1858), 20170380. https://doi.org/10.1098/rspb.2017.0380

Boomsma, J. J., & Have, T. M. V. D. (2002). Queen mating and paternity variation in the ant Lasius niger. *Molecular Ecology*, *7*(12), 1709–1718. https://doi.org/10.1046/j.1365-294x.1998.00504.x

Burda, H., Honeycutt, R. L., Begall, S., Locker-Grütjen, O., & Scharff, A. (2000). Are naked and common mole-rats eusocial and if so, why? *Behavioral Ecology and Sociobiology*, *47*(5), 293–303. https://doi.org/10.1007/s002650050669

Calabi, P., & Porter, S. D. (1989). Worker longevity in the fire ant Solenopsis invicta: Ergonomic considerations of correlations between temperature, size and metabolic rates. *Journal of Insect Physiology*, *35*(8), 643–649. https://doi.org/10.1016/0022-1910(89)90127-3

Capilla-Lasheras, P., Dominoni, D. M., Babayan, S. A., O'Shaughnessy, P. J., Mladenova, M., Woodford, L., … Helm, B. (2017). Elevated Immune Gene Expression Is Associated with Poor Reproductive Success of Urban Blue Tits. *Frontiers in Ecology and Evolution*, *5*. https://doi.org/10.3389/fevo.2017.00064

Carapito, C., Burel, A., Guterl, P., Walter, A., Varrier, F., Bertile, F., & Van Dorsselaer, A. (2014). MSDA, a proteomics software suite for in-depth Mass Spectrometry Data Analysis using grid computing. *Proteomics*, *14*(9), 1014–1019.

Cassill, D. L., Butler, J., Vinson, S. B., & Wheeler, D. E. (2005). Cooperation during prey digestion between workers and larvae in the ant, Pheidole spadonia. *Insectes Sociaux*, *52*(4), 339–343. https://doi.org/10.1007/s00040-005-0817-x

Collado, M., Blasco, M. A., & Serrano, M. (2007). Cellular Senescence in Cancer and Aging. *Cell*, *130*(2), 223–233. https://doi.org/10.1016/j.cell.2007.07.003

Corona, M., Libbrecht, R., Wurm, Y., Riba-Grognuz, O., Studer, R. A., & Keller, L. (2013). Vitellogenin underwent subfunctionalization to acquire caste and behavioral specific expression in the harvester ant Pogonomyrmex barbatus. *PLoS Genet*, *9*(8), e1003730.

Cox, J., Hein, M. Y., Luber, C. A., Paron, I., Nagaraj, N., & Mann, M. (2014). Accurate Proteome-wide Label-free Quantification by Delayed Normalization and Maximal Peptide Ratio Extraction, Termed MaxLFQ. *Molecular & Cellular Proteomics*, *13*(9), 2513–2526. https://doi.org/10.1074/mcp.M113.031591

Cremer, S., Armitage, S. A. O., & Schmid-Hempel, P. (2007). Social Immunity. *Current Biology*, *17*(16), R693–R702. https://doi.org/10.1016/j.cub.2007.06.008

Deursen, J. M. van. (2014). The role of senescent cells in ageing. *Nature*, *509*(7501), 439. https://doi.org/10.1038/nature13193

Domisthorpe, H. (1927). *British ants* (George Routledge and Sons, Ltd.). London.

Duffy, J. E., Morrison, C. L., & R;íos, R. (2000). Multiple origins of eusociality among sponge-dwelling shrimps (synalpheus). *Evolution*, *54*(2), 503–516. https://doi.org/10.1554/0014-3820(2000)054[0503:MOOEAS]2.0.CO;2

Erthal, M., Peres Silva, C., & Ian Samuels, R. (2007). Digestive enzymes in larvae of the leaf cutting ant, Acromyrmex subterraneus (Hymenoptera: Formicidae: Attini). *Journal of Insect Physiology*, *53*(11), 1101–1111. https://doi.org/10.1016/j.jinsphys.2007.06.014

Evans, J. D., Pettis, J. S., & Mueller, U. (2005). Colony-level impacts of immune responsiveness in honey bees, apis mellifera. *Evolution*, *59*(10), 2270–2274. https://doi.org/10.1554/05-060.1

Fang, Y., Song, F., Zhang, L., Aleku, D. W., Han, B., Feng, M., & Li, J. (2012). Differential antennal proteome comparison of adult honeybee drone, worker and queen (Apis mellifera L.). *Journal of Proteomics*, *75*(3), 756–773.

Ferral, N., Holloway, K., Li, M., Yin, Z., & Hou, C. (2017). Heterogeneous activity causes a nonlinear increase in the group energy use of ant workers isolated from queen and brood. *Insect Science*. https://doi.org/10.1111/1744-7917.12433

Feyereisen, R. (1999). Insect P450 Enzymes. *Annual Review of Entomology*, *44*(1), 507–533. https://doi.org/10.1146/annurev.ento.44.1.507

Fjerdingstad, E. J., Gertsch, P. J., & Keller, L. (2002). Why do some social insect queens mate with several males? testing the sex-ratio manipulation hypothesis in lasius niger. *Evolution*, *56*(3), 553–562. https://doi.org/10.1554/0014-3820(2002)056[0553:WDSSIQ]2.0.CO;2

Fjerdingstad, E. J., Keller, L., & Tregenza, T. (2004). Relationships between phenotype, mating behavior, and fitness of queens in the ant lasius niger. *Evolution*, *58*(5), 1056–1063. https://doi.org/10.1554/03-535

Godfrey, S. S., Bull, C. M., Murray, K., & Gardner, M. G. (2006). Transmission mode and distribution of parasites among groups of the social lizard Egernia stokesii. *Parasitology Research*, *99*(3), 223–230. https://doi.org/10.1007/s00436-005-0120-9

Gospocic, J., Shields, E. J., Glastad, K. M., Lin, Y., Penick, C. A., Yan, H., … others. (2017). The neuropeptide corazonin controls social behavior and caste identity in ants. *Cell*, *170*(4), 748–759.

Graeff, J., Jemielity, S., Parker, J. D., Parker, K. M., & Keller, L. (2007). Differential gene expression between adult queens and workers in the ant Lasius niger. *Molecular Ecology*, *16*(3), 675–683.

Grozinger, C. M., Fan, Y., Hoover, S. E., & Winston, M. L. (2007). Genome-wide analysis reveals differences in brain gene expression patterns associated with caste and reproductive status in honey bees (Apis mellifera). *Molecular Ecology*, *16*(22), 4837–4848. https://doi.org/10.1111/j.1365-294X.2007.03545.x

Guidugli, K. R., Nascimento, A. M., Amdam, G. V., Barchuk, A. R., Omholt, S., Simões, Z. L., & Hartfelder, K. (2005). Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Letters*, *579*(22), 4961–4965.

Gygi, S. P., Rochon, Y., Franza, B. R., & Aebersold, R. (1999). Correlation between Protein and mRNA Abundance in Yeast. *Molecular and Cellular Biology*, *19*(3), 1720–1730. https://doi.org/10.1128/MCB.19.3.1720

Harvell, C. D. (1994). The Evolution of Polymorphism in Colonial Invertebrates and Social Insects. *The Quarterly Review of Biology*, *69*(2), 155–185. https://doi.org/10.1086/418538

Have, T. M. van der, Boomsma, J. J., & Menken, S. B. J. (1988). Sex-investment ratios and relatedness in the monogynous ant Lasius niger (l.). *Evolution*, *42*(1), 160–172. https://doi.org/10.1111/j.1558-5646.1988.tb04116.x

Helliwell, S. B., Wagner, P., Kunz, J., Deuter-Reinhard, M., Henriquez, R., & Hall, M. N. (1994). TOR1 and TOR2 are structurally and functionally similar but not identical phosphatidylinositol kinase homologues in yeast. *Molecular Biology of the Cell*, *5*(1), 105–118. https://doi.org/10.1091/mbc.5.1.105

Hölldobler, B., & Wilson, E. O. (1990). *The Ants*. Harvard University Press.

Hunt, J. H., Wolschin, F., Henshaw, M. T., Newman, T. C., Toth, A. L., & Amdam, G. V. (2010). Differential Gene Expression and Protein Abundance Evince Ontogenetic Bias toward Castes in a Primitively Eusocial Wasp. *PLOS ONE*, *5*(5), e10674. https://doi.org/10.1371/journal.pone.0010674

Jeanne, R. L. (1986). The evolution of the organization of work in social insects. *Italian Journal of Zoology*, *20*(2), 119–133. https://doi.org/10.1080/00269786.1986.10736494

Josse, J., & Husson, F. (2016). missMDA: a package for handling missing values in multivariate data analysis. *Journal of Statistical Software*, *70*(1), 1–31.

Judice, C. C., Carazzole, M. F., Festa, F., Sogayar, M. C., Hartfelder, K., & Pereira, G. a. G. (2006). Gene expression profiles underlying alternative caste phenotypes in a highly

eusocial bee, Melipona quadrifasciata. *Insect Molecular Biology*, *15*(1), 33–44. https://doi.org/10.1111/j.1365-2583.2005.00605.x

Judice, C., Hartfelder, K., & Pereira, G. a. G. (2004). Caste-specific gene expression in the stingless bee Melipona quadrifasciata – Are there common patterns in highly eusocial bees? *Insectes Sociaux*, *51*(4), 352–358. https://doi.org/10.1007/s00040-004-0771-z

Jun, J.-I., & Lau, L. F. (2010). The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nature Cell Biology*, *12*(7), 676–685. https://doi.org/10.1038/ncb2070

Kapahi, P., Chen, D., Rogers, A. N., Katewa, S. D., Li, P. W., Thomas, E. L., & Kockel, L. (2010). With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. *Cell Metabolism, Cell Metabolism*, *11*, *11*(6, 6), 453, 453–465. https://doi.org/10.1016/j.cmet.2010.05.001, 10.1016/j.cmet.2010.05.001

Keller, L., & Genoud, M. (1997). Extraordinary lifespans in ants: a test of evolutionary theories of ageing. *Nature*, *389*(6654), 958–960. https://doi.org/10.1038/40130

Khuong, A., Gautrais, J., Perna, A., Sbaï, C., Combe, M., Kuntz, P., … Theraulaz, G. (2016). Stigmergic construction and topochemical information shape ant nest architecture. *Proceedings of the National Academy of Sciences*, *113*(5), 1303–1308. https://doi.org/10.1073/pnas.1509829113

Kohlmeier, P., Feldmeyer, B., & Foitzik, S. (2018). Vitellogenin-like A–associated shifts in social cue responsiveness regulate behavioral task specialization in an ant. *PLOS Biology*, *16*(6), e2005747. https://doi.org/10.1371/journal.pbio.2005747

Kopp, E. B., & Medzhitov, R. (2009). Infection and inflammation in somatic maintenance, growth and longevity. *Evolutionary Applications*, *2*(1), 132–141. https://doi.org/10.1111/j.1752-4571.2008.00062.x

Kriventseva, E. V., Kuznetsov, D., Tegenfeldt, F., Manni, M., Dias, R., Simão, F. A., & Zdobnov, E. M. (2019). OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. *Nucleic Acids Research*, *47*(D1), D807–D811. https://doi.org/10.1093/nar/gky1053

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, *025*(i01). Retrieved from https://ideas.repec.org/a/jss/jstsof/v025i01.html

LeBoeuf, A. C., Waridel, P., Brent, C. S., Gonçalves, A. N., Menin, L., Ortiz, D., … Keller, L. (n.d.). Oral transfer of chemical cues, growth proteins and hormones in social insects. *ELife*, *5*. https://doi.org/10.7554/eLife.20375

Lenoir, A. (1979). *comportement alimentaire et la division du travail chez la fourmi Lasius niger (L.)* (Université de Tours). Retrieved from http://www.cataglyphis.fr/Publis%20AL/Alain%20Lenoir%20Thesis%201979.pdf

Li, J., Wu, J., Rundassa, D. B., Song, F., Zheng, A., & Fang, Y. (2010). Differential protein expression in honeybee (Apis mellifera L.) larvae: underlying caste differentiation. *PLoS One*, *5*(10), e13455.

Libbrecht, R., Corona, M., Wende, F., Azevedo, D. O., Serrão, J. E., & Keller, L. (2013). Interplay between insulin signaling, juvenile hormone, and vitellogenin regulates

maternal effects on polyphenism in ants. *Proceedings of the National Academy of Sciences*, *110*(27), 11050–11055.

López-Uribe, M. M., Sconiers, W. B., Frank, S. D., Dunn, R. R., & Tarpy, D. R. (2016). Reduced cellular immune response in social insect lineages. *Biology Letters*, *12*(3), 20150984. https://doi.org/10.1098/rsbl.2015.0984

Malta, J., Martins, G. F., Marques, A. E., Games, P. D., Zanuncio, J. C., Baracat-Pereira, M. C., & Salomão, T. M. F. (2014). Insights Into the Proteome of the Spermatheca of the Leaf-Cutting Ant Atta sexdens rubropilosa (Hymenoptera: Formicidae). *Http://Dx.Doi.Org/10.1653/024.097.0467*, *101*(3), 1856–1861. https://doi.org/10.1653/024.097.0467

Matjusaitis, M., Chin, G., Sarnoski, E. A., & Stolzing, A. (2016). Biomarkers to identify and isolate senescent cells. *Ageing Research Reviews*, *29*, 1–12. https://doi.org/10.1016/j.arr.2016.05.003

Mersch, D. P., Crespi, A., & Keller, L. (2013). Tracking Individuals Shows Spatial Fidelity Is a Key Regulator of Ant Social Organization. *Science*, *340*(6136), 1090–1093. https://doi.org/10.1126/science.1234316

Mitchell, A. L., Attwood, T. K., Babbitt, P. C., Blum, M., Bork, P., Bridge, A., … Finn, R. D. (2019). InterPro in 2019: improving coverage, classification and access to protein sequence annotations. *Nucleic Acids Research*, *47*(D1), D351–D360. https://doi.org/10.1093/nar/gky1100

Moret, Y., & Schmid-Hempel, P. (2000). Survival for Immunity: The Price of Immune System Activation for Bumblebee Workers. *Science*, *290*(5494), 1166–1168. https://doi.org/10.1126/science.290.5494.1166

Morton Wheeler, W. (1908). The Polymorphism of Ants. *Annals of the Entomological Society of America*, *1*(1), 39–69. https://doi.org/10.1093/aesa/1.1.39

Nelson, C. M., Ihle, K. E., Fondrk, M. K., Page Jr, R. E., & Amdam, G. V. (2007). The gene vitellogenin has multiple coordinating effects on social organization. *PLoS Biol*, *5*(3), e62.

Ong, D. S. T., Wang, L., Zhu, Y., Ho, B., & Ding, J. L. (2005). The response of ferritin to LPS and acute phase of Pseudomonas infection. *Journal of Endotoxin Research*, *11*(5), 267–280. https://doi.org/10.1179/096805105X58698

Oppert, B., Guedes, R. N. C., Aikins, M. J., Perkin, L., Chen, Z., Phillips, T. W., … Arthur, F. (2015). Genes related to mitochondrial functions are differentially expressed in phosphine-resistant and -susceptible Tribolium castaneum. *BMC Genomics*, *16*(1), 968. https://doi.org/10.1186/s12864-015-2121-0

Page, R. E., & Amdam, G. V. (2007). The making of a social insect: developmental architectures of social design. *BioEssays*, *29*(4), 334–343. https://doi.org/10.1002/bies.20549

Parker, J. D. (2010). What are social insects telling us about aging? *Myrmecological News*, *13*, 103–110.

Patel, A., Fondrk, M. K., Kaftanoglu, O., Emore, C., Hunt, G., Frederick, K., & Amdam, G. V. (2007). The Making of a Queen: TOR Pathway Is a Key Player in Diphenic Caste Development. *PLOS ONE*, *2*(6), e509. https://doi.org/10.1371/journal.pone.0000509

Powers, R. W., Kaeberlein, M., Caldwell, S. D., Kennedy, B. K., & Fields, S. (2006). Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes & Development*, *20*(2), 174–184. https://doi.org/10.1101/gad.1381406

Ravikumar, B., Berger, Z., Vacher, C., O'Kane, C. J., & Rubinsztein, D. C. (2006). Rapamycin pre-treatment protects against apoptosis. *Human Molecular Genetics*, *15*(7), 1209–1216. https://doi.org/10.1093/hmg/ddl036

Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annual Review of Entomology*, *37*(1), 637–665.

Robinson, G. E., Grozinger, C. M., & Whitfield, C. W. (2005). Sociogenomics: social life in molecular terms. *Nature Reviews Genetics*, *6*(4), 257–270. https://doi.org/10.1038/nrg1575

Robinson, G. E., Strambi, C., Strambi, A., & Feldlaufer, M. F. (1991). Comparison of juvenile hormone and ecdysteroid haemolymph titres in adult worker and queen honey bees (Apis mellifera). *Journal of Insect Physiology*, *37*(12), 929–935. https://doi.org/10.1016/0022-1910(91)90008-N

Saumweber, T., Weyhersmüller, A., Hallermann, S., Diegelmann, S., Michels, B., Bucher, D., … Gerber, B. (2011). Behavioral and synaptic plasticity are impaired upon lack of the synaptic protein SAP47. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *31*(9), 3508–3518. https://doi.org/10.1523/JNEUROSCI.2646-10.2011

Schmid-Hempel, P. (1998). *Parasites in Social Insects*. Princeton University Press.

Seeley, T. D. (1986). Division of Labour among Worker Honeybees. *Ethology*, *71*(3), 249–251. https://doi.org/10.1111/j.1439-0310.1986.tb00588.x

Skike, C. E. V., & Galvan, V. (2018). A Perfect sTORm: The Role of the Mammalian Target of Rapamycin (mTOR) in Cerebrovascular Dysfunction of Alzheimer's Disease: A Mini-Review. *Gerontology*, (0). https://doi.org/10.1159/000485381

Smirle, M. J., & Winston, M. L. (2011). Detoxifying enzyme activity in worker honey bees: an adaptation for foraging in contaminated ecosystems. *Canadian Journal of Zoology*. https://doi.org/10.1139/z88-283

Stearns, S. C. (1977). The Evolution of Life History Traits: A Critique of the Theory and a Review of the Data. *Annual Review of Ecology and Systematics*, *8*(1), 145–171. https://doi.org/10.1146/annurev.es.08.110177.001045

Sterry, P. (1997). *Collins Complete British Wildlife Photoguide*. HarperCollins.

Sumner, S. (2006). Determining the molecular basis of sociality in insects: progress, prospects and potential in sociogenomics. *Annales Zoologici Fennici*, *43*(5/6), 423–442.

Tan, Q.-Q., Liu, W., Zhu, F., Lei, C.-L., Hahn, D. A., & Wang, X.-P. (2017). Describing the diapause-preparatory proteome of the beetle Colaphellus bowringi and identifying candidates affecting lipid accumulation using isobaric tags for mass spectrometry-based proteome quantification (iTRAQ). *Frontiers in Physiology*, *8*. https://doi.org/10.3389/fphys.2017.00251

Tchkonia, T., Zhu, Y., Deursen, J. van, Campisi, J., & Kirkland, J. L. (2013). Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *The Journal of Clinical Investigation*, *123*(3), 966–972. https://doi.org/10.1172/JCI64098

Vizcaíno, J. A., Csordas, A., del-Toro, N., Dianes, J. A., Griss, J., Lavidas, I., … others. (2016). 2016 update of the PRIDE database and its related tools. *Nucleic Acids Research*, *44*(D1), D447–D456.

Vogt, J. T., & Appel, A. G. (1999). Standard metabolic rate of the fire ant, Solenopsis invicta Buren: effects of temperature, mass, and caste. *Journal of Insect Physiology*, *45*(7), 655–666.

Went, F. W., Wheeler, J., & Wheeler, G. C. (1972). Feeding and Digestion in Some Ants (Veromessor and Manica). *BioScience*, *22*(2), 82–88. https://doi.org/10.2307/1296037

Werck-Reichhart, D., & Feyereisen, R. (2000). Cytochromes P450: a success story. *Genome Biology*, *1*, reviews3003. https://doi.org/10.1186/gb-2000-1-6-reviews3003

Wiese, M. D., Chataway, T. K., Davies, N. W., Milne, R. W., Brown, S. G. A., Gai, W.-P., & Heddle, R. J. (2006). Proteomic analysis of Myrmecia pilosula (jack jumper) ant venom. *Toxicon*, *47*(2), 208–217. https://doi.org/10.1016/j.toxicon.2005.10.018

Willot, Q., Gueydan, C., & Aron, S. (2017). Proteome stability, heat hardening and heat-shock protein expression profiles in Cataglyphis desert ants. *Journal of Experimental Biology*, *220*(9), 1721–1728. https://doi.org/10.1242/jeb.154161

Wilson, E. O. (1971). The insect societies. *The Insect Societies.* Retrieved from https://www.cabdirect.org/cabdirect/abstract/19720503745

Yan, H., Opachaloemphan, C., Mancini, G., Yang, H., Gallitto, M., Mlejnek, J., … Desplan, C. (2017). Olfactory receptors are required for social behavior and neural plasticity in ants, as evidenced by CRISPR-mediated gene knockout. *BioRxiv*, 142232. https://doi.org/10.1101/142232