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Wolbachia prevalence, diversity, and ability to induce cytoplasmic incompatibility in mosquitoes

Mathieu Sicard, Manon Bonneau and Mylène Weill

To protect humans and domestic animals from mosquito borne diseases, alternative methods to chemical insecticides have to be found. Pilot studies using the vertically transmitted bacterial endosymbiont *Wolbachia* were already launched in different parts of the world. *Wolbachia* can be used either in Incompatible Insect Technique (IIT), to decrease mosquito population, or to decrease the ability of mosquitoes to transmit pathogens. Not all mosquito species are naturally infected with *Wolbachia*: while in *Culex pipiens* and *Aedes albopictus* almost all individuals harbor *Wolbachia*, putative infections have to be further investigated in *Anopheles* species and in *Aedes aegypti*. All *Wolbachia*-based control methods rely on the ability of *Wolbachia* to induce cytoplasmic incompatibility (CI) resulting in embryonic death in incompatible crossings. Knowledge on CI diversity in mosquito is required to find the better *Wolbachia*-mosquito associations to optimize the success of both 'sterile insect' and 'pathogen blocking' *Wolbachia*-based methods.

Address
ISEM, University of Montpellier, CNRS, EPHE, IRD, Montpellier, France

Corresponding authors:
Sicard, Mathieu (mathieu.sicard@umontpellier.fr),
Weill, Mylène (mylene.weill@umontpellier.fr)

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Edited by Anna Cohuet and Claudio Lazzari

Introduction

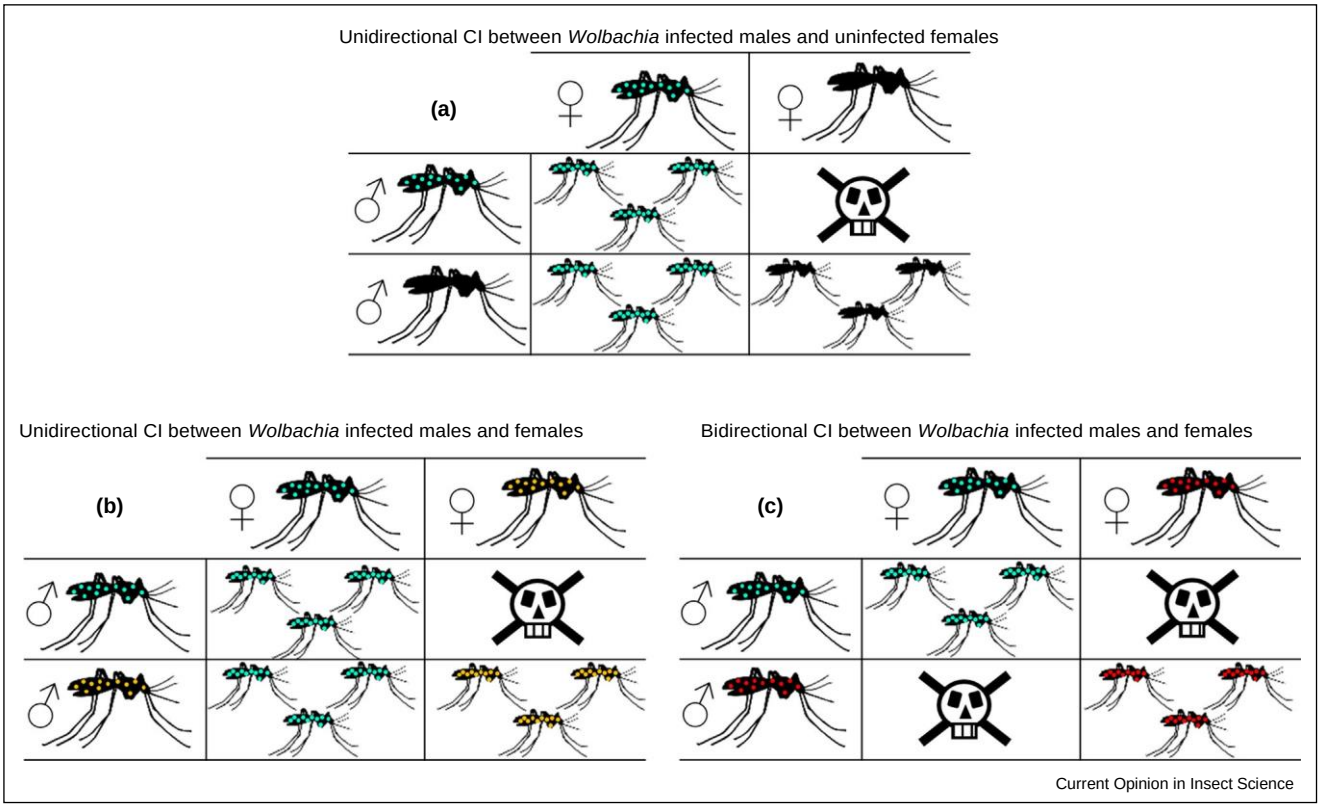
Mosquitoes are vectors for major pathogens such as arboviruses, nematodes and protozoans. To protect humans and domestic animals from these pathogens, strategies targeting the vectors aim at decreasing vector population density and/or at diminishing their ability to transmit pathogens [1••]. Presently, the most common vector control actions are intended to decrease the longevity and the density in vector populations, mainly by using chemical insecticides, which have reached their limits because of genetic resistance and negative consequence on non-targeted invertebrate species [2]. In this context, new vector control strategies, hopefully more environmental friendly, have to be proposed. Biocontrol strategies of vectors based on the knowledge of their microbiota are promising [3], and particularly those based on *α*-proteobacteria of the genus *Wolbachia* which manipulate many aspects of their mosquito host biology [4–6]. Because these symbionts can influence both mosquito reproduction and their pathogen loads, *Wolbachia*-based control methods can be deployed to reduce vector populations and/or to diminish their capacity to transmit pathogens.

The *Wolbachia*, which are maternally transmitted endosymbionts, have long been studied because of their ability to manipulate their host reproduction to increase their prevalence within host populations [4]. Cytoplasmic incompatibility (CI) is the most frequent manipulation used by *Wolbachia* to spread within insect populations [4]. During the invasion process, CI occurs when males infected with a given *Wolbachia* breed with uninfected females which, then, produce non-viable embryos (Figure 1). As crossings between individuals infected with compatible *Wolbachia* give normal viable embryos, the consequence of CI is that the prevalence of *Wolbachia* increases within the host population so that prevalence can reach 100%.

The ability of *Wolbachia* to induce CI is the cornerstone of the two major *Wolbachia*-based control methods developed to date (Box 1). The first method, called 'Incompatible Insect Techniques' (IIT), is related to the classical sterile insect technics (SIT) [1••], and aims at decreasing mosquito population size by releasing *Wolbachia* infected males that are incompatible with local females. In this strategy, the local females produce non-viable embryos resulting locally and temporally in the vector population crash-down [7,8,9••,10]. The second method uses CI induction by *Wolbachia*, not to reduce the density of a focal vector population, but to sustainably replace its uninfected individuals by *Wolbachia* infected ones. Indeed it has been shown that *Wolbachia* can interfere negatively with the transmission of disease pathogens including the major arboviruses Chikungunya, Dengue, Rift Valley, West-Nile, Zika, and so on [11•,12••,13••,14–18,19•]. In this strategy, CI allows the progressive invasion of local vector population with individuals harboring *Wolbachia* which mediate blocking of arboviruses transmission [20–22].

Wolbachia being a promising weapon against mosquitoes, we synthetize in this review the current knowledge

Figure 1



The different types of CI in mosquitoes.
(a) The simplest type of CI occurring between *Wolbachia* infected males and uninfected females allows *Wolbachia* to invade uninfected populations. In *Culex pipiens* and *Aedes albopictus*, *Wolbachia* has reached fixation in nature; this type of CI is, thus, only observed in laboratory conditions when females are artificially cured of their *Wolbachia* with an antibiotic treatment. This type of CI was also observed when *Ae. aegypti* and *An. stephensi* males were transinfected with a *Wolbachia* strain from other species and crossed with naturally uninfected females. (b–c) Other types of CI can occur between males and females both infected with different *Wolbachia* strains. In such crosses CI can be unidirectional (i.e. only one of the reciprocal crosses is compatible, the other is incompatible) (b) or bidirectional (both reciprocal crosses are incompatible) (c). Unidirectional CI can be observed in *Ae. albopictus* when bi-infected males (infected with both *w*AlbA and *w*AlbB) are crossed with laboratory females infected only with *w*AlbA. In *Culex pipiens*, really complex CI crossing types are observed, including unidirectional and bidirectional CI depending on the *w*Pip strains present in crossed individuals.

accumulated on *Wolbachia* prevalence, diversity, and ability to induce CI in the major vector mosquito species of *Aedes*, *Anopheles*, and *Culex* genera.

Prevalence and diversity of *Wolbachia* in mosquitoes

The diversity hidden behind the term '*Wolbachia pipientis*' in arthropod and nematode hosts is presently organized in 17 phylogenetic clades called supergroups (A to Q) [23–27]. Within each supergroup, the unit of diversity is called a 'strain'. Most of the *Wolbachia* strains were named according to their host species (e.g. *w*Pip in *Culex pipiens* and *w*Alb in *Aedes albopictus*). If genetic differences are identified within an already defined 'strain', new information including sampling location or phylogenetic position can be added to name the new strains. The increase in genomic analyses to investigate more and more accurately *Wolbachia* diversity, between and within host species, may lead to important changes in the definition of new 'strains' in the near future [28].

Anopheles mosquitoes, the major vectors of *Plasmodium*, were considered to be exempt of *Wolbachia* because classic PCR diagnostic tests were always found negative [29–31]. However, very deep sequencing of *Wolbachia*-specific 16S rRNA recently suggested putative natural infections of *Anopheles coluzzii* and *Anopheles gambiae* in Burkina Faso [32•,33]. The *Wolbachia* 16S sequences obtained were attributed to a new strain named '*w*Anga'. Positive mothers did not produce only positive offspring ruling out both an insertion in host genome and a perfect vertical transmission of *Wolbachia* [32•]. Such a genetic detection of *Wolbachia* has now been extended to

Box 1 *Wolbachia* anti-vectorial methods: either decrease the density or modify the physiology of mosquitoes

a In the Incompatible Insect Technique (IIT) large numbers of *Wolbachia*-infected males are released. The *Wolbachia* harboring by these males has to be carefully chosen to ensure that these males will be able to kill, due to CI, embryos of females from the focal population. This requires that females either do not harbor the same *Wolbachia* strain (for instance in *Ae. albopictus* and *C. pipiens* cases) or that are putatively not infected with *Wolbachia* (for instance in *Ae. aegypti* and *An. gambiae*). After repeated releases of incompatible males, the vector population will decrease. To be successful, the *Wolbachia* strain in the released males should be involved in bidirectional CI with the *Wolbachia* strain from the targeted population (Figures 1 and 2). This way, the released *Wolbachia* has almost no chance to settle in the introduced environment because even if females are concomitantly released with infected males, they would not be able to produce offspring with the local males. The release of the same line of *Wolbachia* infected males can stay efficient through years. For even more efficiency, ITT may be combined with sterile insect technique (SIT) by irradiating *Wolbachia*-infected mosquitoes.

b *Wolbachia* can also be used to modify the physiology of mosquito. In this method, both *Wolbachia* infected male and female mosquitoes are released. CI allows the progressive invasion of local vector population with individuals harboring *Wolbachia* which mediates blocking of arboviruses transmission. To be successful, the *Wolbachia* of the released individuals have (i) to block viruses and (ii) to exhibit a unidirectional CI relationship with the targeted populations that allows the spread and sustainability of protective *Wolbachia* (Figure 2).

Anopheles funestus from Senegal [34], *Anopheles arabiensis* in Tanzania [35], 16 *Anopheles* species among a total of 25 in Gabon [36], and in 5 species among 17 from Ghana, Democratic Republic of the Congo (DRC), Guinea, Uganda and Madagascar [37]. *Wolbachia* 16S sequences exhibited much larger diversity than usually expected within one strain suggesting multiple infections. Indeed, these sequences can be clustered with those from *Wolbachia* strains belonging to the supergroups A, B and even, more surprisingly, to supergroup C [36,38••]. The reported proportions of positive individuals vary among species and localities but remain low for most of the *Anopheles* species. Besides the interesting case of *Anopheles moucheti* in Gabon and DRC, for which the prevalence of *Wolbachia* seems very high, calls for further studies [36,37]. The low detection of *Wolbachia* in most *Anopheles* species could be due to a low prevalence of the symbiont that required more individuals and screened populations to be detected. However, if *Wolbachia* are really present in these *Anopheles* species their density must be very low as several screening technics revealed discordant results, even requiring nested PCR or quantitative PCR with a very high number of amplification cycles for detection [34]. The main problem is that the putative presence of *Wolbachia* in all these *Anopheles* species is mostly based on its genetic detection which is not an actual proof of real infection and could result from contaminations, at least for certain species [38••]. To our knowledge, no electronic microscopy observations that would provide a direct proof of infections, have been yet conducted. Only one study reported fluorescent *in situ* hybridization (FISH) labelling to monitor the presence of *Wolbachia*, found at low density in the ovaries of some *An. coluzzii* [33].

Aedes aegypti and *Ae. albopictus*, the major arboviruses vectors, although belonging to the same genus exhibit strongly different patterns in terms of *Wolbachia* infection. As for *Anopheles*, classic PCR tests were always negative on *Ae. aegypti* placing this species among uninfected ones. However, deep sequencing of *Wolbachia*-specific 16S rRNA from both larvae and adults in USA and Thailand were recently found positive, indicating the putative presence of *Wolbachia* in some individuals [39,40]. Nevertheless, as it is the case with the *Anopheles*, if *Wolbachia* cells are present in this vector it must be at low prevalence and at a 'cryptic' load [41]. Further investigations including symbiont visualization must be conducted in the future to confirm the presence of *Wolbachia* at low prevalence and titer in *Ae. aegypti*.

In contrast, *Ae. albopictus* is found infected with *Wolbachia* everywhere in the world [42,43]. All individuals are usually infected with two *Wolbachia* strains namely *w*AlbA and *w*AlbB belonging to the supergroups A and B, respectively [44]. However, a polymorphism of the infection status exists: (i) *w*AlbB mono-infected males (but not females) have been reported in La Réunion Island and Madagascar field populations [45], and (ii) *w*AlbA mono-infected laboratory lines were obtained from individuals initially sampled in Thailand and Mauritius [46,47]. The genetic variation within both *w*AlbA and *w*AlbB strains is yet considered to be low as no variation was detected within each strain based on 16S rRNA, wsp and ftsZ gene sequences [42,46–48] suggesting that *Wolbachia* could have recently, invaded and spread throughout populations of this mosquito species to finally reach fixation [42].

In *C. pipiens (s.l.)*, all individuals are infected with *w*Pip *Wolbachia* that were also found non-polymorphic using MLST genes [23,24]. However, MLSTs including a larger number of highly polymorphic genes (MutL, ank2, pk1, pk2, GP12, GP15, and RepA) allowed to uncover a previously hidden diversity [49]. All *w*Pip strains are monophyletic and closely related, and they form five groups from *w*Pip-I to *w*Pip-V. As the thousands *C. pipiens* individuals tested around the world [50–53] harbored a *w*Pip strain belonging to one of the five groups, the infection is considered to have reached fixation in this species. However, few individuals in South Africa, France, Scotland and Tunisia were found negative to *Wolbachia* genetic tests [54,55]. A phylogenetic analysis based on mitochondrial markers demonstrated that all these uninfected mosquitoes form a new species named '

Culex juppi nov. sp.' independent from all the infected *C. pipiens* [55].

CI induction in natural *Wolbachia*-mosquito associations

In *Anopheles* no cytoplasmic incompatibility has been shown in laboratory crosses between males putatively infected with *Wolbachia* and uninfected females [33]. Such laboratory observations are in accordance with the low detection of the symbionts in *Anopheles* natural populations. However, an acceleration of egg laying in *Wolbachia* positive females has been reported [33]. In *Ae. albopictus*, both *w*AlbA and *w*AlbB were reported to increase host fecundity [8]. CI does not occur between individuals from lines originating from distant parts of the world since most individuals are bi-infected with *w*AlbA and *w*AlbB showing no or low polymorphism [43] (Figure 1). Consequently, there is only one dominant crossing type in *Ae. albopictus* natural populations all over the world, resulting in compatibility between all lines. Nevertheless, females mono-infected with only *w*AlbA strain produce unviable embryos when crossed with normally bi-infected males resulting in unidirectional CI [46,47] (Figure 1). This clearly demonstrated that *w*AlbB strain is able to induce CI but that this CI phenotype rarely occurs in nature because of the high frequency of bi-infections with *w*AlbA and *w*AlbB.

In contrast to the absence of CI recorded in *Anopheles* and the poor crossing type diversity observed in *Ae. albopictus*, the hundreds of crosses performed between *C. pipiens* lines sampled worldwide have revealed an unrivaled diversity of crossing types [56,57•,58] (Figure 1). Genetic diversity within the *w*Pip clade is responsible for this unique CI polymorphism since (i) no other manipulative endosymbiont was detected in this host species, (ii) the host genetic background did not influence the crossing types, and more importantly (iii) *C. pipiens* lines harboring *w*Pip belonging to the same phylogenetic group (*w*Pip-I–V) are generally compatible, whereas 'inter-group crosses' are more likely to be incompatible [57•]. Infected males harboring a *w*Pip strain (from any *w*Pip group) induce total CI (i.e. no embryo will develop) when crossed with uninfected females while the reciprocal crossing is fertile [59–61,62•]. Such unidirectional CI pattern between uninfected and infected individuals has certainly prevailed during the spread of the *w*Pip infection in *C. pipiens* populations, but is no longer observed in the wild since infection reached fixation. To date, crosses can only occur between (i) individuals infected with the same *w*Pip group (usually resulting in normal reproduction) or (ii) individuals harboring *w*Pip from two different groups. Such 'inter-group crossings' can have three outcomes (Figure 1): (i) production of living offspring; (ii) unidirectional CI (one cross direction is compatible while the reciprocal one is incompatible) or (iii) bidirectional CI (both cross directions are incompatible).

CI induction in artificial *Wolbachia*-mosquito associations

When *Wolbachia* have been experimentally introduced by transinfection in two 'non-infected mosquito species' namely *Ae. aegypti* and *Anopheles stephensi*, CI has been observed showing that *Wolbachia* molecular targets responsible for CI are present in these species. Indeed, *Ae. aegypti* has been successfully transinfected independently with eight *Wolbachia* strains (*w*Mel, *w*MelPop-CLA, *w*MelCS, *w*Ri, *w*Au, *w*AlbA, *w*AlbB, and *w*Pip [14,19•,63–65]) (Figure 2); and all induced unidirectional CI with natural uninfected *Ae. aegypti* except *w*Au which is a *Wolbachia* strain from *D. simulans* that also does not induce CI in its natural host [19•]. *An. stephensi* has also been successfully transinfected with *w*AlbB from *Ae. albopictus* which induced CI enabling *Wolbachia* to invade uninfected laboratory populations [66]. Transinfections have also been conducted in *Ae. albopictus*, which is naturally infected, in order to create new crossing types. Both *w*Pip and *w*Mel strains have been introduced in *Wolbachia*-cured lines resulting in bidirectional incompatibility between transinfected lines and naturally infected ones [67–69]. Moreover, a triple-infected (*w*AlbA, *w*AlbB, and *w*Pip) *Ae. albopictus* line has been established; it expresses unidirectional CI when crossed with naturally double-infected mosquitoes.

C. pipiens has not yet been transinfected with other *Wolbachia* since the natural crossing type diversity demonstrated in this species can provide with unidirectional and bidirectional crossing types required in *Wolbachia*-based control methods (e.g. [53]).

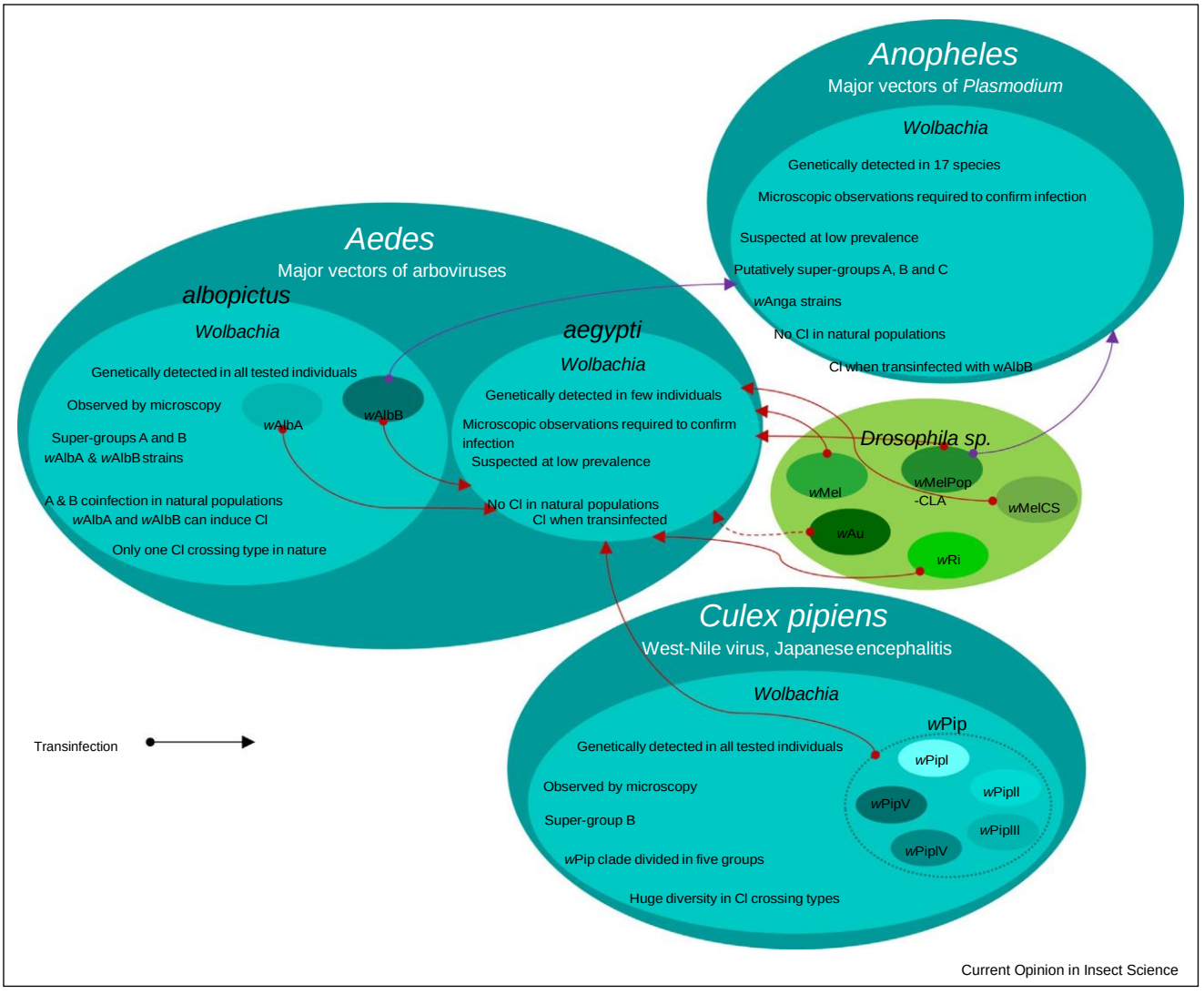
Cellular mechanism of CI in mosquitoes

The cellular mechanism of CI has only been yet studied in details in *C. pipiens* [62•]. To do so, the early embryogenesis was monitored using fluorescence confocal microscopy in (i) fertile intra-group crosses, (ii) incompatible crosses between infected males and infected females (i.e. inter-group crosses), and (iii) incompatible crosses between infected males and uninfected females. Despite the diversity of the crosses involving various *w*Pip strains, common embryonic defects resulting in the death of the embryos were detected. These defects consisted in paternal chromatin condensation and segregation impairments during the first embryonic division as for *Drosophila* and *Nasonia* [62•,70–74] (Figure 3).

Wolbachia genes involved in CI in mosquitoes

Cytological observations in *C. pipiens* suggest that a toxin, deposited in maturing sperm, would prevent the development of embryos by impairing paternal

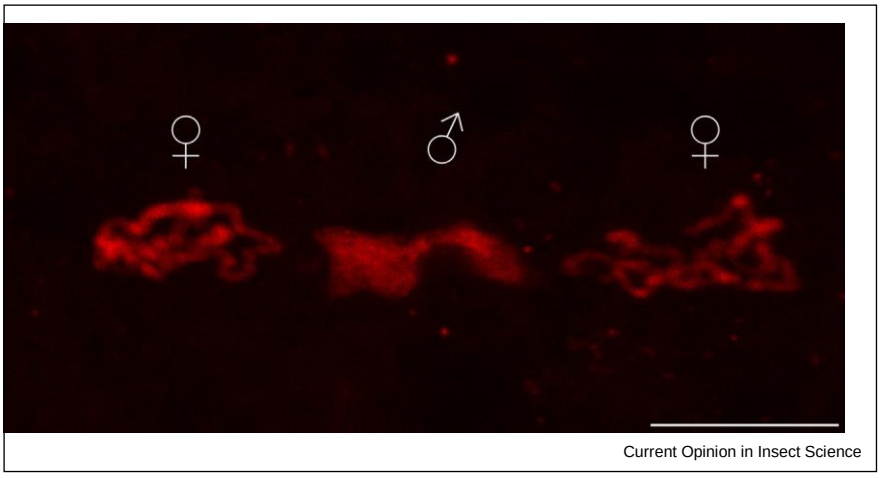
Figure 2



A synthetic view of *Wolbachia* knowledge in mosquitoes.
Arrows represent *Wolbachia* transinfection from one mosquito donor species to a recipient mosquito species or from *Drosophila* to a mosquito recipient species. The *Anopheles* transinfected species was *An. stephensi* [66]. Solid arrows represent strains that were able to induce CI when transferred to a recipient host while the dashed arrow represent the strain *w*Au that does not induce CI.

chromatin normal segregation unless they are rescued by an antidote [75]. A combination of approaches on different insects demonstrated that the *Wolbachia cidA* and *cidB* genes, first identified by the presence of CidA protein in *C. pipiens* sperm [76], were the determinant in the induction and rescue of CI in insects [77••,78••]. Biochemical analyses revealed that CidB protein could act as toxin since it encodes a putatively toxic deubiquitylase (DUB). Convincingly, when *cidA* and *cidB* were transgenically expressed in uninfected *Drosophila* males, these males were incompatible with uninfected females: embryos were unviable, and the first embryonic mitosis displayed the same characteristics as in CI [77••,78••]. CidA is most probably the antidote against the toxic activity of CidB since its expression during early oogenesis restored the viability of uninfected eggs fertilized by *Drosophila* infected males [79]. Both *cidA* and *cidB* genes are monomorphic in *w*AlbB. No genomic data are yet available on *w*AlbA and on the putative strain *w*Anga from *Anopheles*. However, in *C. pipiens*, these genes are amplified and diversified within each *w*Pip genome constituting the fuel for the diversity of crossing types described in this species [80•]. This *cidA/cidB* gene amplifications and diversifications in *w*Pip may also account for the impressive CI penetrance observed in *C. pipiens* [62•].

Figure 3



CI cellular phenotype as observed after the first embryonic division in *Culex pipiens*.
Paternal chromatin and maternal chromatin were labelled using propidium iodide and were observed under confocal microscopy. In the picture, we can see that paternal chromatin (<) failed to segregate during the first mitotic embryonic division while maternal chromatin (,) did segregate. Because of this defect in the first division, the embryos will not be able to develop normally into larvae. Scale bar is 10 *m*m.

Conclusion

Prevalence and diversity of *Wolbachia* are quite contrasted between mosquito species. *Ae. albopictus* and *C. pipiens* individuals all harbor diverse *Wolbachia* that can induce CI and influence their life history traits at each generation. In contrast, the major arboviruses vector *Ae. aegypti* and the major malaria vectors, *Anopheles* spp., are only suspected to be infected. Further studies are required to investigate infection status of these last species. Recent studies on *C. pipiens* along with those conduced on *Drosophila* brought new elements on CI mechanisms, both at cellular and molecular levels that constitute the cornerstone for an efficient use of *Wolbachia* genetic resources in vector control.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Flores HA, O'Neill SL: Controlling vector-borne diseases by
•• releasing modified mosquitoes. *Nat Rev Microbiol* 2018, 16:508-518.
This review focused on new vector control methods based on the release of genetically modified or *Wolbachia*-infected mosquitoes.

2. Labbé P, David JP, Alout H, Milesi P, Djogbénou L, Pasteur N, Weill M: Evolution of resistance to insecticide in disease vectors. In *Genetics and Evolution of Infectious Diseases,* edn 2. Edited by Tibayrenc M. Elsevier Inc.; 2017:313-339.

3. Ricci I, Valzano M, Ulissi U, Epis S, Cappelli A, Favia G: Symbiotic control of mosquito borne disease. *Pathog Glob Health* 2012, 106:380-385.

4. Werren JH, Baldo L, Clark ME: *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol* 2008, 6:741-751.

5. Zug R, Hammerstein P: Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. *Biol Rev* 2015, 90:89-111.

6. Sicard M, Dittmer J, Grève P, Bouchon D, Braquart-Varnier C: A host as an ecosystem: *Wolbachia* coping with environmental constraints. *Environ Microbiol* 2014, 16:3583-3607.

7. Laven H: Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature* 1967, 216:383-384.

8. Dobson SL, Fox CW, Jiggins FM: The effect of *Wolbachia*-induced cytoplasmic incompatibility on host population size in natural and manipulated systems. *Proc R Soc B Biol Sci* 2002, 269:437-445.

9. Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C,
•• Bourtzis K: *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proc Natl Acad Sci U S A* 2004, 101:15042-15045.
The results presented in this study constituted the cornerstone to encourage methods based on the introduction of *Wolbachia* into pest and vector species of economic and health relevance to suppress or modify natural populations.

10. Zabalou S, Apostolaki A, Livadaras I, Franz G, Robinson AS, Savakis C, Bourtzis K: Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain. *Entomol Exp Appl* 2009, 132:232-240.

11. • Hedges LM, Brownlie JC, O'Neill SL, Johnson KN: *Wolbachia* and virus protection in insects. *Science* 2008, 322:702.
In this study, the 'protective effect' of *Wolbachia* against the *Drosophila* C virus *in Drosophila melanogaster* was revealed. The infected flies showed a delay in mortality compared to flies cured of *Wolbachia* infection. These results indicated that *Wolbachia* could exhibit an antiviral effect.

12. Teixeira L, Ferreira Á, Ashburner M: The bacterial symbiont
•• *Wolbachia* induces resistance to RNA viral infections in *Drosophila melanogaster*. *PLoS Biol* 2008, 6:2753-2763.
In this study, the 'protective effects' of *Wolbachia* against RNA viruses (*Drosophila* C virus, Nora viruse and Flock House virus) and DNA virus

(Insect Iridescent virus 6) in *Drosophila melanogaster* were analyzed. 'Protective effects' were found in RNA viruses but not with one DNA virus.

13. •• Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M *et al.*: A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and *Plasmodium*. *Cell* 2009, 139:1268-1278.

This study showed that *Wolbachia* infection directly inhibits the ability of a range of pathogens to infect *Aedes aegypti.* The effect is *Wolbachia* strain-specific and relates to *Wolbachia* priming of the mosquito innate immune system and potentially competition for limiting cellular resources required for pathogen replication.

14. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, Leong YS, Dong Y, Axford J, Kriesner P *et al.*: The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 2011, 476:450-455.

15. Blagrove MSC, Arias-Goeta C, Failloux A-B, Sinkins SP: *Wolbachia* strain *w*Mel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. *Proc Natl Acad Sci U S A* 2012, 109:255-260.

16. Dutra HLC, Rocha MN, Dias FBS, Mansur SB, Caragata EP, Moreira LA: *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. *Cell Host Microbe* 2016, 19:771-774.

17. Aliota MT, Peinado SA, Velez ID, Osorio JE: The *w*Mel strain of *Wolbachia* reduces transmission of Zika virus by *Aedes aegypti*. *Sci Rep* 2016, 6:1-7.

18. Aliota MT, Walker EC, Uribe Yepes A, Dario Velez I, Christensen BM, Osorio JE: The *w*Mel strain of *Wolbachia* reduces transmission of Chikungunya virus in *Aedes aegypti*. *PLoS Negl Trop Dis* 2016, 10:e0004677.

19. • Ant TH, Herd CS, Geoghegan V, Hoffmann AA, Sinkins SP: The *Wolbachia* strain *w*Au provides highly efficient virus transmission blocking in *Aedes aegypti*. *PLoS Pathog* 2018, 14:e1006815.

In this study, the 'protective effects' of several *Wolbachia* strains transinfected in the *Aedes aegypti* mosquito against arboviruses (Semliki Forest, Dengue and Zika) were analyzed. Among these *Wolbachia* strains, the *w*Au strain from *Drosophila simulans* provided highly efficient transmission blocking of arboviruses. However, *w*Au did not induce CI and must be associated with a CI-inducing strain such as *w*AlbB from *Aedes albopictus* to be used in vector-control strategies.

20. Frentiu FD, Zakir T, Walker T, Popovici J, Pyke AT, van den Hurk A, McGraw EA, O'Neill SL: Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. *PLoS Negl Trop Dis* 2014, 8:1-10.

21. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y *et al.*: Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 2011, 476:454-457.

22. Hoffmann AA, Iturbe-Ormaetxe I, Callahan AG, Phillips BL, Billington K, Axford JK, Montgomery B, Turley AP, O'Neill SL: Stability of the *w*Mel *Wolbachia* infection following invasion into *Aedes aegypti* populations. *PLoS Negl Trop Dis* 2014, 8:e3115.

23. Baldo L, Hotopp JCD, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MCJ, Tettelin H, Werren JH: Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl Environ Microbiol* 2006, 72:7098-7110.

24. Paraskevopoulos C, Bordenstein SR, Wernegreen JJ, Werren JH, Bourtzis K: Toward a *Wolbachia* multilocus sequence typing system: discrimination of *Wolbachia* strains present in *Drosophila* species. *Curr Microbiol* 2006, 53:388-395.

25. Bordenstein SR, Paraskevopoulos C, Dunning Hotopp JC, Sapountzis P, Lo N, Bandi C, Tettelin H, Werren JH, Bourtzis K: Parasitism and mutualism in *Wolbachia*: what the phylogenomic trees can and cannot say. *Mol Biol Evol* 2009, 26:231-241.

26. Ros VID, Fleming VM, Feil EJ, Breeuwer JAJ: How diverse is the genus *Wolbachia*? Multiple-gene sequencing reveals a putatively new *Wolbachia* supergroup recovered from spider mites (Acari: Tetranychidae). *Appl Environ Microbiol* 2009, 75:1036-1043.

27. Glowska E, Dragun-Damian A, Dabert M, Gerth M: New *Wolbachia* supergroups detected in quill mites (Acari: Syringophilidae). *Infect Genet Evol* 2015, 30:140-146.

28. Turelli M, Cooper BS, Richardson KM, Ginsberg PS, Peckenpaugh B, Antelope CX, Kim KJ, May MR, Abrieux A, Wilson DA *et al.*: Rapid global spread of *w*Ri-like *Wolbachia* across multiple *Drosophila*. *Curr Biol* 2018, 28:963-971.e8.

29. Jamnongluk W, Kittayapong P, Baisley KJ, O'Neill SL: *Wolbachia* infection and expression of cytoplasmic incompatibility in *Armigeres subalbatus* (Diptera: Culicidae). *J Med Entomol* 2000, 37:53-57.

30. Ricci I, Cancrini G, Gabrielli S, D'amelio S, Favia G: Searching for *Wolbachia* (Rickettsiales: Rickettsiaceae) in mosquitoes (Diptera: Culicidae): large polymerase chain reaction survey and new identifications. *J Med Entomol* 2002, 39:562-567.

31. Rasgon JL, Scott TW: An initial survey for *Wolbachia* (Rickettsiales: Rickettsiaceae) infections in selected California mosquitoes (Diptera: Culicidae): Table 1. *J Med Entomol* 2004, 41:255-257.

32. • Baldini F, Segata N, Pompon J, Marcenac P, Robert Shaw W, Dabiré RK, Diabaté A, Levashina EA, Catteruccia F: Evidence of natural *Wolbachia* infections in field populations of *Anopheles gambiae*. *Nat Commun* 2014, 5:3985.

This study suggested for the first time putative *Wolbachia* infections in *Anopheles gambiae* long time thought as not infected with *Wolbachia*.

33. Shaw WR, Marcenac P, Childs LM, Buckee CO, Baldini F, Sawadogo SP, Dabiré RK, Diabaté A, Catteruccia F: *Wolbachia* infections in natural *Anopheles* populations affect egg laying and negatively correlate with *Plasmodium* development. *Nat Commun* 2016, 7:11772.

34. Niang EHA, Bassene H, Makoundou P, Fenollar F, Weill M, Mediannikov O: First report of natural *Wolbachia* infection in wild *Anopheles funestus* population in Senegal. *Malar J* 2018, 17:1-6.

35. Baldini F, Rougé J, Kreppel K, Mkandawile G, Mapua SA, Sikulu-Lord M, Ferguson HM, Govella N, Okumu FO: First report of natural *Wolbachia* infection in the malaria mosquito *Anopheles arabiensis* in Tanzania. *Parasites Vectors* 2018, 11:635.

36. Ayala D, Akone-Ella O, Rahola N, Kengne P, Ngangue MF, Mezeme F, Makanga BK, Costantini C, Simard F, Prugnolle F *et al.*: Natural *Wolbachia* infections are common in the major malaria vectors in Central Africa. *bioRxiv* 2018 http://dx.doi.org/10.1101/343715.

37. Jeffries CL, Lawrence GG, Golovko G, Kristan M, Orsborne J, Spence K, Hurn E, Bandibabone J, Tantely LM, Raharimalala FN *et al.*: Novel *Wolbachia* strains in *Anopheles* malaria vectors from Sub-Saharan Africa. *Wellcome Open Res* 2018, 3:113.

38. •• Chrostek E, Gerth M: Is *Anopheles gambiae* a natural host of *Wolbachia*? *bioRxiv* 2018 http://dx.doi.org/10.1101/491449.

The *Wolbachia* infection status of *Anopheles gambiae* was questioned in this study as the evidences of *Wolbachia* infection in this species have unusual properties: high *Wolbachia* 16S sequence diversity, low abundance and lack of congruence between hosts. The authors believe that the unusual characteristics of *Wolbachia* sequences render them insufficient to diagnose *Wolbachia* infection and that sequence-independent evidence such as microscopic observation are required to confirm the infection.

39. Coon KL, Brown MR, Strand MR: Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. *Mol Ecol* 2016, 25:5806-5826.

40. Thongsripong P, Chandler JA, Green AB, Kittayapong P, Wilcox BA, Kapan DD, Bennett SN: Mosquito vector-associated microbiota: metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. *Ecol Evol* 2018, 8:1352-1368.

41. Schneider DI, Riegler M, Arthofer W, Merçot H, Stauffer C, Miller WJ: Uncovering *Wolbachia* diversity upon artificial host transfer. *PLoS One* 2013, 8:e82402.

42. Armbruster P, Damsky WE, Giordano R, Birungi J, Munstermann LE, Conn JE: Infection of new- and old-world *Aedes albopictus* (Diptera: Culicidae) by the intracellular parasite *Wolbachia*: implications for host mitochondrial DNA evolution. *J Med Entomol* 2003, 40:356-360.

43. Bourtzis K, Dobson SL, Xi Z, Rasgon JL, Calvitti M, Moreira LA, Bossin HC, Moretti R, Baton LA, Hughes GL *et al.*: Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. *Acta Trop* 2014, 132:S150-S163.

44. Zhou W, Rousset F, O'Neill S: Phylogeny and PCR–based classification of *Wolbachia* strains using *wsp* gene sequences. *Proc R Soc Lond Ser B Biol Sci* 1998, 265:509-515.

45. Tortosa P, Charlat S, Labbé P, Dehecq J-S, Barré H, Weill M: *Wolbachia* age-sex-specific density in *Aedes albopictus*: a host evolutionary response to cytoplasmic incompatibility? *PLoS One* 2010, 5:e9700.

46. Kambhampati S, Rai KS, Burgun SJ: Unidirectional cytoplasmic incompatibility in the mosquito, *Aedes albopictus*. *Evolution (N Y)* 1993, 47:673-677.

47. Sinkins S, Braig H, O'neill S: *Wolbachia* superinfections and the expression of cytoplasmic incompatibility. *Proc Biol Sci* 1995, 261:325-330.

48. de Albuquerque AL, Magalhães T, Ayres CFJ: High prevalence and lack of diversity of *Wolbachia pipientis* in *Aedes albopictus* populations from Northeast Brazil. *Mem Inst Oswaldo Cruz* 2011, 106:773-776.

49. Atyame CM, Delsuc F, Pasteur N, Weill M, Duron O: Diversification of *Wolbachia* endosymbiont in the *Culex pipiens* mosquito. *Mol Biol Evol* 2011, 28:2761-2772.

50. Dumas E, Atyame CM, Milesi P, Fonseca DM, Shaikevich EV, Unal S, Makoundou P, Weill M, Duron O: Population structure of *Wolbachia* and cytoplasmic introgression in a complex of mosquito species. *BMC Evol Biol* 2013, 13:181.

51. Atyame CM, Labbé P, Rousset F, Beji M, Makoundou P, Duron O, Dumas E, Pasteur N, Bouattour A, Fort P *et al.*: Stable coexistence of incompatible *Wolbachia* along a narrow contact zone in mosquito field populations. *Mol Ecol* 2015, 24:508-521.

52. Atyame CM, Pasteur N, Dumas E, Tortosa P, Tantely ML, Pocquet N, Licciardi S, Bheecarry A, Zumbo B, Weill M *et al.*: Cytoplasmic incompatibility as a means of controlling *Culex pipiens quinquefasciatus* mosquito in the islands of the South-Western Indian Ocean. *PLoS Negl Trop Dis* 2011, 5:e1440.

53. Atyame CM, Cattel J, Lebon C, Flores O, Dehecq J-S, Weill M, Gouagna LC, Tortosa P: *Wolbachia*-based population control strategy targeting *Culex quinquefasciatus* mosquitoes proves efficient under semi-field conditions. *PLoS One* 2015, 10:e0119288.

54. Rasgon JL, Scott TW: *Wolbachia* and cytoplasmic incompatibility in the California *Culex pipiens* mosquito species complex: parameter estimates and infection dynamics in natural populations. *Genetics* 2003, 165:2029-2038.

55. Dumas E, Atyame CM, Malcolm CA, Le Goff G, Unal S, Makoundou P, Pasteur N, Weill M, Duron O: Molecular data reveal a cryptic species within the *Culex pipiens* mosquito complex. *Insect Mol Biol* 2016, 25:800-809.

56. Laven H: Speciation and evolution in *Culex pipiens*. In *Genetics of Insect Vectors of Disease*. Edited by Wright J, Pal R. Amsterdam: Elsevier; 1967:251-275.

57. • Atyame CM, Labbé P, Dumas E, Milesi P, Charlat S, Fort P, Weill M: *Wolbachia* divergence and the evolution of cytoplasmic incompatibility in *Culex pipiens*. *PLoS One* 2014, 9:e87336.

In this study, the genetic diversity of *w*Pip strains in the mosquito *Culex pipiens* and the CI phenotypes induced by these strains were analyzed showing a significant association of *Wolbachia* genetic divergence and CI phenotypes: most *w*Pip strains from the same *w*Pip group were compatible whereas those from different *w*Pip groups were often incompatible.

58. Atyame CM, Duron O, Tortosa P, Pasteur N, Fort P, Weill M: Multiple *Wolbachia* determinants control the evolution of cytoplasmic incompatibilities in *Culex pipiens* mosquito populations. *Mol Ecol* 2011, 20:286-298.

59. Yen JH, Barr AR: The etiological agent of cytoplasmic incompatibility in *Culex pipiens*. *J Invertebr Pathol* 1973, 22:242-250.

60. Duron O, Weill M: *Wolbachia* infection influences the development of *Culex pipiens* embryo in incompatible crosses. *Heredity (Edinb)* 2006, 96:493-500.

61. Duron O, Fort P, Weill M: Influence of aging on cytoplasmic incompatibility, sperm modification and *Wolbachia* density in *Culex pipiens* mosquitoes. *Heredity (Edinb)* 2007, 98:368-374.

62. • Bonneau M, Landmann F, Labbé P, Justy F, Weill M, Sicard M: The cellular phenotype of cytoplasmic incompatibility in *Culex pipiens* in the light of *cidB* diversity. *PLoS Pathog* 2018, 14: e1007364.

In this study, the cellular mechanism of CI induced by *Wolbachia* was first time studied in a mosquito (*Culex pipiens*) demonstrating similar defects than those observed in *Drosophila* and *Nasonia*.

63. McMeniman CJ, Roxanna LV, Bodil CN, Amy FWC, Manpreet S, Yu-Feng W, O'Neill SL: Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science (80-)* 2009, 323:141-144.

64. Xi Z, Khoo CCH, Dobson SL: *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science (80-)* 2005, 310:326-328.

65. Fraser JE, De Bruyne JT, Iturbe-Ormaetxe I, Stepnell J, Burns RL, Flores HA, O'Neill SL: Novel *Wolbachia*-transinfected *Aedes aegypti* mosquitoes possess diverse fitness and vector competence phenotypes. *PLoS Pathog* 2017, 13:1-19.

66. Bian G, Joshi D, Dong Y, Lu P, Zhou G, Pan X, Xu Y, Dimopoulos G, Xi Z: *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. *Science (80-)* 2013, 340:748-751.

67. Blagrove MSC, Arias-Goeta C, Di Genua C, Failloux A-B, Sinkins SP: A *Wolbachia w*Mel transinfection in *Aedes albopictus* is not detrimental to host fitness and inhibits Chikungunya virus. *PLoS Negl Trop Dis* 2013, 7:e2152.

68. Calvitti M, Moretti R, Lampazzi E, Bellini R, Dobson SL: Characterization of a new *Aedes albopictus* (Diptera: Culicidae)-*Wolbachia pipientis* (Rickettsiales: Rickettsiaceae) symbiotic association generated by artificial transfer of the *w*Pip strain from *Culex pipiens* (Diptera: Culicida). *J Med Entomol* 2010, 47:179-187.

69. Calvitti M, Marini F, Desiderio A, Puggioli A, Moretti R: *Wolbachia* density and cytoplasmic incompatibility in *Aedes albopictus*: concerns with using artificial *Wolbachia* infection as a vector suppression tool. *PLoS One* 2015, 10:1-19.

70. Tram U, Sullivan W: Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility. *Science* 2002, 296:1124-1126.

71. Tram U, Fredrick K, Werren JH, Sullivan W: Paternal chromosome segregation during the first mitotic division determines *Wolbachia*-induced cytoplasmic incompatibility phenotype. *J Cell Sci* 2006, 119:3655-3663.

72. Landmann F, Orsi GA, Loppin B, Sullivan W: *Wolbachia*-mediated cytoplasmic incompatibility is associated with impaired histone deposition in the male pronucleus. *PLoS Pathog* 2009, 5:e1000343.

73. Callaini G, Riparbelli MG, Giordano R, Dallai R: Mitotic defects associated with cytoplasmic incompatibility in *Drosophila simulans*. *J Invertebr Pathol* 1996, 67:55-64.

74. Callaini G, Dallai R, Riparbelli MG: *Wolbachia*-induced delay of paternal chromatin condensation does not prevent maternal chromosomes from entering anaphase in incompatible crosses of *Drosophila simulans*. *J Cell Sci* 1997, 110:271-280.

75. Beckmann JF, Bonneau M, Chen H, Hochstrasser M, Poinsot D, Mercot H, Weill M, Sicard M, Charlat S: The toxin-antidote model of cytoplasmic incompatibility: genetics and evolutionary implications. *Trends Genet*, in press.

76. Beckmann JF, Fallon AM: Detection of the *Wolbachia* protein WPIP0282 in mosquito spermathecae: implications for cytoplasmic incompatibility. *Insect Biochem Mol Biol* 2013, 43:867-878.

77. Beckmann JF, Ronau JA, Hochstrasser M: A *Wolbachia* •• deubiquitylating enzyme induces cytoplasmic incompatibility. *Nat Microbiol* 2017, 2:17007.

In this paper, the capacity of the genes *cidA* and *cidB* from the *Wolbachia* *w*Pip to induce CI when transgenetically expressed in *Drosophila melanogaster* was studied. *D. melanogaster* males expressing both *cidA* and *cidB* genes were able to induce CI when crossed with uninfected females, demonstrating the implication of these two genes in CI induction.

78. LePage DP, Metcalf JA, Bordenstein SR, On J, Perlmutter JI, •• Shropshire JD, Layton EM, Funkhouser-Jones LJ, Beckmann JF, Bordenstein SR: Prophage WO genes recapitulate and enhance *Wolbachia*-induced cytoplasmic incompatibility. *Nature* 2017, 543:243-247.

In this study, the capacity of *cifA* and *cifB* genes from the *Wolbachia* *w*Mel genome to induce CI when transgenetically expressed in *Drosophila melanogaster* was analyzed. *D. melanogaster* males expressing both *cifA* and *cifB* were able to induce CI when crossed with uninfected female while males expressing either *cifA* or *cifB* genes could not. Infected females were fertile with transgenic males showing the ability of the *Wolbachia* of rescuing the effect of *cifA* and *cifB* genes.

79. Shropshire JD, On J, Layton EM, Zhou H, Bordenstein SR: One prophage WO gene rescues cytoplasmic incompatibility in *Drosophila melanogaster*. *Proc Natl Acad Sci U S A* 2018, 115:4987-4991.

80. Bonneau M, Atyame C, Beji M, Justy F, Cohen-Gonsaud M, • Sicard M, Weill M: *Culex pipiens* crossing type diversity is governed by an amplified and polymorphic operon of *Wolbachia*. *Nat Commun* 2018, 9:319.

This study demonstrated the presence of several different *cidA* and *cidB* variants in each *w*Pip strain genomes studied and the association of specific *cidA* and *cidB* variants with a given CI phenotype strongly supporting the implication of these genes in the unrivaled CI diversity described in *Culex pipiens*.