



***Wolbachia* infections in *Drosophila melanogaster* and *D. simulans*: polymorphism and levels of cytoplasmic incompatibility**

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Abstract

Wolbachia are endosymbiotic bacteria, widespread in terrestrial Arthropods. They are mainly transmitted vertically, from mothers to offspring and induce various alterations of their hosts' sexuality and reproduction, the most commonly reported phenomenon being Cytoplasmic Incompatibility (CI), observed in *Drosophila melanogaster* and *D. simulans*. Basically, CI results in a more or less intense embryonic mortality, occurring in crosses between males infected by *Wolbachia* and uninfected females. In *D. simulans*, *Wolbachia* and CI were observed in 1986. Since then, this host species has become a model system for investigating the polymorphism of *Wolbachia* infections and CI. In this review we describe the different *Wolbachia* infections currently known to occur in *D. melanogaster* and *D. simulans*. The two species are highly contrasting with regard to symbiotic diversity: while five *Wolbachia* variants have been described in *D. simulans* natural populations, *D. melanogaster* seems to harbor one *Wolbachia* variant only. Another marked difference between these two *Drosophila* species is their permissiveness with regard to CI, which seems to be fully expressed in *D. simulans* but partially or totally repressed in *D. melanogaster*, demonstrating the involvement of host factors in the control of CI levels. The potential of the two host species regarding the understanding of CI and its evolution is also discussed.

Introduction

Wolbachia are endosymbiotic bacteria from the Rickettsiaceae family (O'Neill et al., 1992; Roux & Raoult, 1995). Four main clades can be distinguished. *Wolbachia* infecting Arthropods all belong to the A and B clades, which form together a monophyletic group (Werren, Zhang & Guo, 1995). Clades C and D include *Wolbachia* from filarial Nematodes (Bandi, Trees & Brattig, 2001). Two additional lineages of *Wolbachia* infecting Arthropods (clades E and F) have also been described, but seem much less diversified (Vandekerckhove et al., 1999; Lo et al., 2002). Although these symbionts are transmitted vertically in both Arthropods and Nematodes, *Wolbachia* and host phylogenies are congruent in Nematodes only (Bandi et al., 1998), suggesting that horizontal transfers oc-

cur between Arthropod hosts (Werren, Zhang & Guo, 1995; Casiraghi et al., 2001).

Wolbachia are present in most tissues, including ovaries and testes (Dobson et al., 1999) but are transmitted by females only, through the egg cytoplasm. In Arthropods, they can induce various alterations of host sexuality and reproduction, namely feminization, thelytokous parthenogenesis, male-killing and Cytoplasmic Incompatibility (CI), all having in common that they will increase the frequency of infected females, allowing *Wolbachia* to invade host populations (reviewed in O'Neill, Hoffmann & Werren, 1997; Werren, 1997; Stouthamer, Breeuwer & Hurst, 1999; Hurst & Jiggins, 2000; Charlat, Bourtzis & Merçot, 2001). CI seems to be the most common *Wolbachia*-induced phenotype, and occurs in *Drosophila melanogaster* and *D. simulans*. In its simplest form, this

phenomenon can be seen when males bearing the bacterium mate with uninfected females. Embryos resulting from such crosses die because chromosomes of paternal origin behave abnormally at the first mitosis (Callaini et al., 1996; Lassy & Karr, 1996; Callaini, Dallai & Ripardelli, 1997; Tram & Sullivan, 2002). By contrast, crosses involving infected females (male uninfected \times female infected and male infected \times female infected) are normally fertile, as well as that between uninfected males and uninfected females. In other words, infected females are immune from the sterility caused by infected males, so that infection frequency increases.

The molecular bases of CI are currently unknown, but the current framework is that of the modification/rescue formalization (Werren, 1997), emphasizing that two aspects of CI must be distinguished: one occurring in the male germline, before *Wolbachia* is shed from maturing sperm (termed *mod*, for modification) that disrupts paternal chromosomes behavior and one occurring in the female germline (termed *resc*, for rescue) that restores normal embryonic development. This model allows to distinguish four different phenotypic categories, based on the expression or extinction of the *mod* and *resc* functions: (i) [*mod+* *resc+*] when *Wolbachia* induces CI and rescues from its own effect, (ii) [*mod-* *resc-*] when *Wolbachia* does not induce CI and is also unable to rescue from the [*mod+*] effect of other variants, (iii) [*mod-* *resc+*] when *Wolbachia* does not induce CI but is capable of rescuing the [*mod+*] effect of another variant and finally (iv) [*mod+* *resc-*] when *Wolbachia* induces CI but is unable to rescue it. As detailed in this article, the three first classes have been found to exist in *Drosophila*. On the contrary, the last type (the 'suicidal' *Wolbachia*) has never been observed, although theory does not preclude its existence (Charlat & Merçot, 2001). It is notable that different *Wolbachia* variants expressing a [*mod+* *resc+*] phenotype can be incompatible with each other. This case is named bi-directional incompatibility (O'Neill & Karr, 1990). In other words, embryonic mortality can occur in crosses between infected males and infected females, if the two partners bear different *Wolbachia*. This demonstrates that the *mod* and *resc* functions interact in a specific manner. Attempts have been made to translate *mod* and *resc* into more concrete factors (Kose & Karr, 1995; Callaini, Dallai & Ripardelli, 1997; Tram & Sullivan, 2002). To our opinion, the *Lock-and-Key* model, assuming a physical interaction between *mod* (the *Lock*) and *resc* (the *Key*)

is the most satisfactory (Poinsot, Charlat & Merçot, 2003).

A bit of history

The discovery of *Wolbachia* dates back to 1924, when Hertig and Wolbach observed them in reproductive cells of the mosquito *Culex pipiens* (Hertig & Wolbach, 1924). The bacterium received its official name (*Wolbachia pipientis*) and description 12 years later (Hertig, 1936). *C. pipiens* was also the host species in which CI was first reported, and found to be maternally inherited (Ghelelovich, 1952). In *C. pipiens* again, *Wolbachia* was finally recognized as the causal agent of CI (Yen & Barr, 1971, 1973).

With regard to *Drosophila*, the bacterium was first observed in *D. melanogaster*. It was variously referred to as 'A bodies' in ovarian cells (King & Mills, 1962; King, 1970), 'granules' (Peacock & Erickson, 1964) or 'Epsilon granule' in the pole cells (Ullmann, 1965). Wolstenholme (1965) gave a full description of what he called 'cytoplasmic DNA and RNA containing bodies', without identifying *Wolbachia* or mentioning any phenotypic effect such as CI. Later, Rickettsia bacteria were identified in spermatocytes (Yanders et al., 1968; Erickson & Acton, 1969) or in cell cultures (Szollosi & Debec, 1980). In the genus *Drosophila*, CI was first reported, in *D. simulans* (Hoffmann, Turelli & Simmons, 1986; Hoffmann & Turelli, 1988) and unambiguously linked to a *Wolbachia*-like organism (Binnington & Hoffmann, 1989). The phenomenon was observed 2 years later in *D. melanogaster* (Hoffmann, 1988), but here the levels of embryonic mortality were much lower than in *D. simulans*. Since then, Polymerase Chain Reaction and sequencing allowed to identify definitively *Wolbachia* in both species (O'Neill et al., 1992; Rousset, Vautrin & Solignac, 1992; Holden, Jones & Brookfield, 1993; Bourtzis et al., 1994). Subsequent investigations have revealed important contrasts between these two hosts, both regarding the diversity of *Wolbachia* infections and their phenotypic effects.

Diversity of *Wolbachia* infections

D. simulans

To date, five *Wolbachia* variants have been described in *D. simulans* natural populations, illustrating

three different phenotypic categories: [*mod+* *resc+*], [*mod-* *resc+*] and [*mod-* *resc-*]. Each of them is associated with one of the three distinct mitochondrial haplotypes (*siI*, *siII* or *siIII*), and their geographic distribution follows that of these haplotypes (see M. Solignac, this issue).

Three variants express a [*mod+* *resc+*] phenotype in their natural host: *w*Ri, *w*Ha and *w*No. Originally discovered in California (Riverside) (Hoffmann, Turelli & Simmons, 1986), the *w*Ri variant belongs to the A clade. It is associated to the *siII* mitochondrial haplotypes and can be found in most continental populations (Africa, Europe, Australia, America). It induces a strong CI, and shows high invasion capabilities (Hoffmann, Turelli & Harshman, 1990; Turelli & Hoffmann, 1991; Turelli, Hoffmann & McKechnie, 1992). Also falling within the A clade, the *w*Ha variant (discovered in Hawaii) was first described by O'Neill and Karr (1990). It is associated to the *siI* haplotype and found in populations from indo-pacific islands (Hawaii, French Polynesia, Seychelles archipelago, New Caledonia) (Montchamp-Moreau, Ferveur & Jacques, 1991; Merçot et al., 1995; Rousset & Solignac, 1995). Finally, the *w*No variant (originating from Nouméa, New Caledonia) belongs to the B clade. It is associated to the *siI* haplotype and seems to be limited to the Seychelles archipelago and New Caledonia where it occurs in bi-infection with *w*Ha (Merçot et al., 1995; Rousset & Solignac, 1995). In natural populations, individuals singly infected by *w*No are very rare (James et al., 2002) although segregation experiments allow to isolate this variant in the laboratory (Merçot et al., 1995; Merçot & Poinso, 1998a; Poinso, Montchamp-Moreau & Merçot, 2000). Finally these three [*mod+* *resc+*] are bi-directionally incompatible.

The two other variants (*w*Ma and *w*Au) do not generally appear to induce CI ([*mod-*] phenotype). *w*Ma belongs to the B group and is associated to the *siIII* haplotype. It was initially described in a population from Madagascar (Nigro, 1991; Rousset & Solignac, 1995) and since then rediscovered in La Réunion Island (James & Ballard, 2000) and down Mount Kilimanjaro (Merçot & Poinso 1998b; Charlat, Le Chat & Merçot, 2003). From this latter population it was shown that *w*Ma still possesses the ability to rescue the CI effect of its close relative *w*No ([*resc+*] phenotype) (Merçot & Poinso 1998b; Poinso & Merçot 1999; Charlat et al., 2002). Let us mention however a puzzling pattern reported by James and Ballard (2000), where *w*Ma-infected males from Mad-

agascar and La Réunion where found to express CI in some, but not all experiments. *w*Au belongs to the A group and is associated to the *siII* haplotype. Initially described in Australia (Hoffmann, Clancy & Ducan, 1996), this variant also occurs in Madagascar (James & Ballard, 2000), West Africa (Charlat, Le Chat & Merçot, 2003) and probably in Ecuador (Turelli & Hoffmann, 1995) although molecular characterization is lacking for this last population. In studies where this trait was investigated, *w*Au was not found to induce CI (Hoffmann, Clancy & Ducan, 1996; James & Ballard, 2000; Reynolds & Hoffmann, 2002; Charlat, Le Chat & Merçot, 2003) and *w*Au does not appear to rescue the *mod* function of all CI inducing strains tested so far (Poinso et al., 1998). However, it must be kept in mind that this [*resc-*] status cannot be considered as definitive: the existence of a compatible [*mod+*] *Wolbachia*, not tested so far, is not ruled out. In connection with that, intriguing results were obtained using two lines from Florida (Ballard et al., 1996). Here the *Wolbachia* infection was found to induce significant CI, although at a low level (less than 20% embryonic mortality), and to rescue itself. Later sequencing results performed on the same lines suggested that *w*Au was responsible for this phenotype (James & Ballard, 2000). Thus in some populations *w*Au might display a [*mod+* *resc+*] phenotype. Nevertheless this last point would deserve further investigations.

D. melanogaster

Wolbachia infections in *D. melanogaster* and their phenotypic effects have been well characterized, both in the field and in laboratory experiments (Hoffmann, Clancy & Merton, 1994; Solignac, Vautrin & Rousset, 1994; Hoffmann, Hercus & Dagher, 1998; Bourtzis et al., 1998; Olsen, Reynolds & Hoffmann, 2001; Reynolds & Hoffmann, 2002; Weeks, Reynolds & Hoffmann, 2002). The infection can be found throughout the geographic range of the species (Solignac, Vautrin & Rousset, 1994), with about 34% of studied isofemale or plurifemale lines being infected. Molecular data suggest that infection in all these populations derive from a unique variant (namely *w*Mel). However sequences of the *wsp* gene, more variable than the previously used loci (16S rRNA and *ftsZ*), reveal that several clones can be distinguished, with one or two polymorphic sites (Zhou, Rousset & O'Neill, 1998). In this species, CI does not seem to be expressed in the field, although it can be detected in the laboratory,

especially if very young males are used (Reynolds & Hoffmann, 2002).

wMel does not infect any other *Drosophila* species, but it is very closely related to the *wAu* variant from *D. simulans*. Yet, after injection from *D. melanogaster* into *D. simulans*, the CI induced by *wMel* was not found rescued by *wAu* (Poinsot et al., 1998), unlike previously stated (Bourtzis et al., 1998) and unfortunately quoted (James & Ballard, 2000).

Infections in other species from the *Melanogaster* subgroup

Among the seven other species from the *Melanogaster* subgroup, two only are not known to be infected: *D. orena* and *D. erecta* (Rousset, 1993; Bourtzis et al., 1996). *Wolbachia* variants within the five other species are more or less related to those observed in *D. simulans*.

D. sechellia

This species is endemic from the Seychelles archipelago. It is known to carry two *Wolbachia* variants (*wSh* and *wSn*) closely related to *wHa* and *wNo*, respectively (Rousset & Solignac, 1995; Zhou, Rousset & O'Neill, 1998; Charlat et al., 2002). Rousset and Solignac (1995) report that two *wSn* subtypes (namely *wSn1* and *wSn2*) can be distinguished, which differ by one substitution at the 16S rRNA locus. Just as *D. simulans* populations from this area, *D. sechellia* is doubly infected (Rousset & Solignac, 1995) but segregation can also occur (Charlat, Bonnavion & Merçot, 2003). Together with the fact that the bi-infection of the two species are associated with closely related mitochondrial haplotypes (namely, the *siI* haplotype in *D. simulans* and the *se* haplotype in *D. sechellia*), this parallel between the two species led to suggest that the double infection was existing prior to the speciation event (Rousset & Solignac, 1995), thought to have occurred around half a million years ago (Hey & Kliman, 1993). This view is based on two assumptions, the validity of which remaining an open question: (i) the split between the *siI* and *se* mitochondrial haplotypes actually reflects the *D. simulans*/*D. sechellia* speciation (that is, subsequent introgression did not occur) and (ii) *Wolbachia* infections were not horizontally transferred between the two species after speciation.

D. mauritiana

Due to a recent introgression event, some populations of *D. mauritiana* bear the *siIII* mitochondrial haplotype of *D. simulans* (Solignac & Monnerot, 1986; Ballard 2000a). Accordingly, these populations bear a *wMa*-like variant (Rousset & Solignac, 1995), most likely transferred from *D. simulans* to *D. mauritiana* together with mitochondria. To avoid confusions, it is however preferable to note this infection as *wMau* instead of *wMa*. Injections into *D. simulans* revealed that just as *wMa* (Merçot & Poinsot, 1998b), *wMau* is capable of rescuing the CI induced by *wNo* (Bourtzis et al., 1998) although it does not induce CI itself (Giordano, O'Neill & Robertson, 1995; Rousset & Solignac, 1995).

D. santomea, *D. teissieri* and *D. Yakuba* (the *Yakuba* complex)

These three species from tropical Africa seem to be infected by the same *Wolbachia*, very closely related to the *D. simulans wAu* variant (based on *wsp* sequences and CI phenotypes) (Lachaise et al., 2000; K. Bourtzis and the present authors, unpublished results). A probable explanation for this distribution is that horizontal transfers occurred recently.

Cytoplasmic incompatibility levels

In *D. simulans*, embryonic mortality in crosses between *Wolbachia*-infected males and uninfected females can range from 0 to 100%. Whether the [*mod*-] phenotype (illustrated by *wMa* and *wAu*) is due to a sudden and complete loss of the *mod* function, or represents one extremity of a quantitative continuum (controlled by bacterial and/or host genes) remains questionable. Quantitative variations of CI levels between the three different [*mod*+] variants are observed (Figure 1). Complete CI (i.e., total sterility of *Wolbachia*-infected males mated with uninfected females) seems rather rare, and occurs only for *wRi*. *wHa* induces a lower CI and is followed by *wNo*. Although intra-type variations seem to occur, especially for *wHa* (Charlat et al., 2002), the different *Wolbachia* variants in *D. simulans* express distinct CI levels. These differences are known to be caused by bacterial rather than host factors, since they can be observed within different host genetic backgrounds (Rousset & de Stordeur, 1994; Merçot & Poinsot, 1998a).

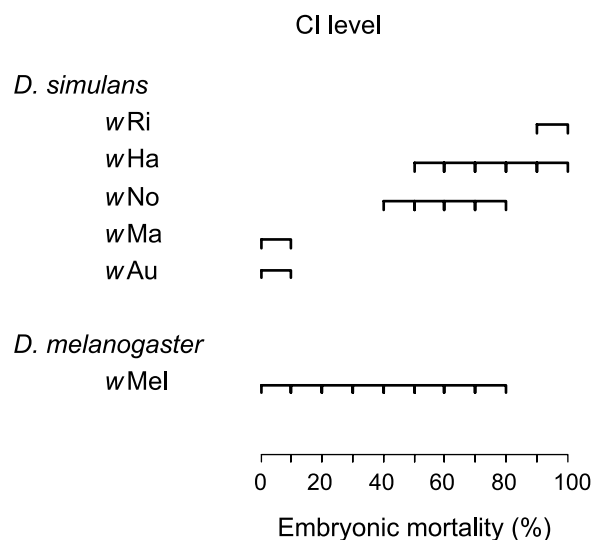


Figure 1. Variations of embryonic mortality observed in crosses between infected males and uninfected females (laboratory conditions). wRi: Hoffmann, Turelli and Simmons (1986), Montchamp-Moreau, Ferveur and Jacques (1991), Giordano, O'Neill and Robertson (1995), Merçot et al. (1995), Bourtzis et al. (1996), and James and Ballard (2000). wHa: O'Neill and Karr (1990), Giordano, O'Neill and Robertson (1995), Merçot et al. (1995), Rousset and Solignac (1995), Bourtzis et al. (1996), and James and Ballard (2000). wNo: Merçot et al. (1995), Merçot and Poinot (1998a), and Poinot and Merçot (1999). wMa: Rousset and Solignac (1995), and Merçot and Poinot (1998b). wAu: Hoffmann, Clancy and Ducan (1996), Merçot and Poinot (1998a), and Poinot et al. (1998). wMel: Hoffmann, Clancy and Merton (1994), Hoffmann, Clancy and Ducan (1996), and Solignac, Vautrin and Rousset (1994).

In *D. melanogaster*, important variations of CI levels have also been observed (Hoffmann, Clancy & Merton, 1994; Solignac, Vautrin & Rousset, 1994; Bourtzis et al., 1998; Hoffmann, Hercus & Dagher, 1998; Reynolds & Hoffmann, 2002). Aside from the field versus laboratory effects, it is unclear if variations between experiments reflect the existence of different wMel clones or an intraspecific variation of the genetic background. Clearly, *D. melanogaster* can lower CI levels, as showed by two independent injection experiments between *D. simulans* and *D. melanogaster*. Boyle et al. (1993) transferred wRi from *D. simulans* into *D. melanogaster*. In its natural host, wRi induced near 100% CI. By contrast, embryonic mortality was only 30% in *D. melanogaster*. Through selection experiments, the authors were subsequently able to obtain higher levels of CI in *D. melanogaster* (80%), correlated with an increase of bacterial density in the eggs. It is unknown whether this increase was due to selection of host and/or bacterial genes

determining higher levels of CI (possibly linked to an increase of bacterial density in the eggs), or to selection of high bacterial density, heritable but not due to genetic variance. Bacterial and host genetic variance being very small after injection experiments, the second explanation is more likely. Poinot et al. (1998) injected wMel (noted as wDm in this publication) from *D. melanogaster* into *D. simulans*. While in the donor *D. melanogaster* line CI level was about 30%, it jumped close to 100% in *D. simulans* injected lines in a few generations. These authors did not observe any correlation between CI levels and densities in the eggs or in males (total body). By contrast, it appeared that CI levels were correlated with the proportion of infected sperm cysts. In the *D. simulans* trans-infected line under study, more than 80% of sperm cysts were found infected, versus 8% only in the donor *D. melanogaster* line. It thus appears that *D. melanogaster* strongly represses the expression of CI, possibly through a control of *Wolbachia* development in the testes.

It is tempting to speculate on a possible relationship between the diversity of *Wolbachia* infections in *D. simulans* and *D. melanogaster* and their respective permissiveness regarding the expression of CI. Infection dynamics models, predict that when *Wolbachia* transmission from mothers to offspring is lower than 100% (which is most often the case), or if infection is somewhat reducing the fitness of infected females, *Wolbachia* cannot invade uninfected host populations unless its frequency first exceeds a threshold value (reviewed in Hoffmann & Turelli, 1997). The lower the level of CI induced, the higher this threshold frequency will be. Leaving aside the puzzling question of why CI repression has evolved in *D. melanogaster* and not in *D. simulans*, the low diversity of infections in *D. melanogaster* can be seen as a consequence of CI repression that strongly reduces invasion probability. Of course, other factors, such as transmission rates or fitness effects might also be relevant. However, the two species do not seem to differ radically regarding these two parameters.

Essential contributions

We will now summarize the main contributions of these two *Drosophila* species to our current knowledge of *Wolbachia*-induced CI. The study of *D. simulans* has allowed some major discoveries. Among these, we should mention in particular: (i) the correlation

between the CI levels and the proportion of infected sperm cysts (Bressac & Rousset, 1993), (ii) the existence of bi-infections (Rousset & Solignac, 1995), (iii) the additive CI effects of *Wolbachia* infections when present in multiple infections (Merçot et al., 1995; Rousset, Braig & O'Neill, 1999), (iv) the possible segregation of two variants among the offspring of a bi-infected mother (Merçot et al., 1995; Poinso, Montchamp-Moreau & Merçot, 2000) and (v) the existence of the [*mod*− *resc*−] (Hoffmann, Clancy & Ducan, 1996) and [*mod*− *resc*+] phenotypes (Bourtzis et al., 1998; Merçot & Poinso, 1998b), suggesting that the *mod* and *resc* functions are genetically distinct (Poinso & Merçot, 1999; Poinso, Charlat & Merçot, 2003). It is also in *D. simulans* that the dynamics of an invading new variant have been studied successfully in the field (*w*Ri, in California: Turelli & Hoffmann, 1991, 1995; Turelli, Hoffmann & McKechnie, 1992). This invasion allowed to show the selective sweep caused by *Wolbachia* on mitochondrial polymorphism, due to their shared transmission mode (Turelli, Hoffmann & McKechnie, 1992; Ballard et al., 1996; Ballard 2000b). It also appears that *D. simulans* has been often subject to successful artificial cytoplasmic injections experiments, either between different host strains within the species (Rousset & de Stordeur, 1994; Sinkins et al., 1995; Rousset, Braig & O'Neill, 1999; Poinso & Merçot, 2001), between closely related species (Giordano, O'Neill & Robertson, 1995; Poinso et al., 1998) or between distant species (Braig et al., 1994). Even a variant inducing thelytokous parthenogenesis in a parasitoid wasp has been transferred into *D. simulans*, but infection was not maintained for more than three generations (Van Meer & Stouthamer, 1999). Finally, important insights into the mechanism of CI came from *D. simulans*, where the most complete cytological observations have been made (Kose & Karr, 1995; Callaini et al., 1996; Callaini, Dallai & Ripardelli, 1997; Lassy & Karr, 1996). Taking over from the pioneering species *C. pipiens*, *D. simulans* now appears as one of the model host for the understanding of CI.

Notable insights also came from *D. melanogaster*. Using this host species, it was shown (i) that host genetic background can greatly affect the expression of CI, as suggested by injections experiments (ii) that *Wolbachia* variants which are not apparently closely related can be partially compatible as appears to be the case between *w*Mel and *w*Ri (Poinso et al., 1998), (iii) that a *Wolbachia* clone (namely, the 'pop-corn'

variant, a subtype of *w*Mel) can induce strongly deleterious effects to its host, at least in the laboratory host strain in which it was found (Min & Benzer, 1997), (iv) that levels of CI can greatly vary between the field and the laboratory (Hoffmann, Clancy & Merton, 1994; Solignac, Vautrin & Rousset, 1994; Hoffmann, Hercus & Dagher, 1998; Bourtzis et al., 1998; Reynolds & Hoffmann, 2002; Weeks, Reynolds & Hoffmann, 2002).

Let us finally sketch a few perspectives about the future of these two *Drosophila* species, as far as *Wolbachia* research is concerned. Many different CI-inducing *Wolbachia* variants have been identified so far on the basis of molecular evidence, but it is not known if this diversity translates into a similar diversity of compatibility types (i.e., if all the different *Wolbachia* variants are bi-directionally incompatible. We currently have no idea if the possible number of compatibility types is virtually infinite or fixed to some practical limit. One possible mean to investigate this issue would be to create in *D. simulans* a sort of '*Wolbachia* bank', where the relationships between numerous variants could be investigated within a single host genetic background. A possible impediment to this project is that *Wolbachia* infections from foreign hosts might be difficult to maintain in some cases. Although *D. simulans* seems, in general, a host where transmission efficiency and CI levels are elevated, this is not always the case (Riegler et al., submitted). Now, when it comes to studying the genetics behind the host factors affecting CI levels, *D. melanogaster* will clearly be the host of choice. First, because such host effects occur in this species, and also because molecular tools will be of great help. Not surprisingly, *w*Mel has been chosen for currently running genome sequencing projects.

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