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Wolbachia injection from usual to naive host in *Drosophila simulans* (Diptera: Drosophilidae)

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Abstract. *Wolbachia pipientis* (Hertig) (Rickettsiaceae) is an endocellular bacterium infecting numerous species of arthropods. The bacterium is harboured by males and females but is only transmitted maternally because spermatocytes shed their *Wolbachia* during maturation. The presence of this endosymbiont can lead to feminisation of the host, parthenogenesis, male-killing or reproductive incompatibility called cytoplasmic incompatibility (CI). Although *Wolbachia* transmission is exclusively maternal, phylogenetic evidence indicates that very rare inter-species transmission events have taken place. Horizontal transmission is possible in the laboratory by transferring cytoplasm from infected to uninfected eggs. Using this technique, we have artificially infected lines of the fruit fly *Drosophila simulans* Sturtevant (Drosophilidae). Recipient lines came from two different *D. simulans* populations. One ("naive" host) is not infected in the wild. The other ("usual" host) is a population naturally carrying *Wolbachia* in the wild. In this second case, recipient flies used in the experiment came from a stock culture that had been cured off its infection beforehand by an antibiotic treatment. Infected *D. simulans* laboratory stocks were used as donors. We assessed the three following parameters: (i) trans-infection success rate (ratio of infected over total female zygote having survived the injection), (ii) level of cytoplasmic incompatibility expressed by trans-infected males three generations post-trans-infection, and (iii) infection loss rate over time in trans-infected lines (percentage of lines having lost the infection after 20 to 40 generations). We observed that parameter (i) did not differ significantly whether the recipient line came from a "naive" or a "usual" host population. However, both (ii) and (iii) were significantly higher in the "naive" trans-infected stock, which is in agreement with earlier theoretical considerations.

INTRODUCTION

Wolbachia are endocellular bacteria infecting arthropods (Werren, 1997). They are found in germinal, as well as in somatic tissues, where they live in intra-cytoplasmic vacuoles, and it is estimated (Werren et al., 1995a) that more than 16% of insect species are infected (within a given species, a population can be entirely infected, polymorphic for the infection, or entirely uninfected). Although *Wolbachia* are harboured by male and female hosts, transmission only occurs maternally through the cytoplasm of the egg, because sperm cells shed their bacteria during maturation (Binnington & Hoffmann, 1989).

The infection can result in various alterations of sexuality and reproduction such as feminisation (Rigaud, 1997), thelytokous parthenogenesis (Stouthamer, 1997), male killing (Hurst et al., 1999) and cytoplasmic incompatibility (Hoffmann & Turelli, 1997). All these phenomena will increase the production of infected cytoplasmic lines.

The most common phenomenon, cytoplasmic incompatibility (CI), is an embryonic mortality induced by the presence of *Wolbachia* during the maturation of male reproductive cells. CI occurs when the *Wolbachia* strain present in the male is absent in the egg. Then, fertilisation is apparently normal but subsequent mitoses are disrupted leading to the death of the zygote (Callaini et al., 1996). This occurs when the female is uninfected (Hoffmann et al., 1986) or when the *Wolbachia* strain harboured by the female is different from that of the male (Breeuwer & Werren, 1990; O'Neill & Karr, 1990). Since the eggs laid by uninfected females are not protected from CI, the fitness of uninfected individuals is lowered, and the infection is expected to spread in an initially uninfected population.

The strict vertical transmission of *Wolbachia* does not hold when considering long evolutionary periods. Molecular data indicate that very closely related *Wolbachia* can be found in hosts as distant as insects and crustacean isopods (Werren et al., 1995b; Zhou et al., 1998). The large and repeated discrepancies between the phylogenies of hosts and those of their *Wolbachia* demonstrate that inter-species transmission (and therefore horizontal transmission) has occurred repeatedly in the past. Moreover, it is possible to transfer *Wolbachia* from one species to another through micro-injection experiments in fertilised eggs (Boyle et al., 1993; Braig et al., 1994; Clancy & Hoffmann, 1997; Poinsot et al., 1998).

We have used the same experimental approach to conduct intra-species transfer of *Wolbachia* using *Drosophila simulans* both as a donor and as a recipient host. The

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recipients were of two types. One type, the "naive host", came from a natural *D. simulans* population that is entirely uninfected in the wild. The other type, the "usual host", came from a natural population that is entirely infected. In this second case the eggs used as recipients came from a laboratory stock that had been artificially cured off its infection beforehand by an antibiotic treatment. Two different *Wolbachia* variants were used in the experiments, and they were injected either separately (using mono-infected *D. simulans* donor stocks) or together (using a bi-infected donor stock). We assessed the three following parameters: (i) trans-infection success rate (ratio of infected over total female zygotes having survived the injection), (ii) level of cytoplasmic incompatibility expressed by trans-infected males three generations post-trans-infection, and (iii) infection loss rate in trans-infected lines (percentage of lines having spontaneously lost the infection after 18–20 months of routine laboratory rearing).

MATERIAL AND METHODS

Wolbachia strains

The two *Wolbachia* strains studied are *w*Ha and *w*No (Rousset & Solignac, 1995). They are known only in populations from indo-pacific islands and are bidirectionally incompatible (Merçot et al., 1995).

Drosophila simulans strains

Donors. R1A, NHa and R3A derive from a naturally infected population (Nouméa, New Caledonia). In the wild, Nouméa individuals are either infected simultaneously by *w*Ha and *w*No, or mono-infected by *w*Ha. The laboratory strain R1A only harbours bi-infected individuals. NHa is a Nouméa stock that was found in the laboratory to be infected by *w*Ha only. R3A was obtained by substituting the Nouméa genome by that of SimO for 11 generations. It was then found to be infected by *w*No only (Merçot et al., 1995).

Recipients. SimO is a naturally uninfected strain from Nar'allah, Tunisia (Montchamp-Moreau et al., 1991). It is used as a recipient in the trans-infection experiment into a naive host. Females from the standard SimO stock are also used in test crosses to detect the expression of CI in males. R1ATC is an uninfected stock derived from R1A by an antibiotic treatment (tetracycline) which cured its *Wolbachia* infection (Poinsot & Merçot, 1997). The asymbiotic status of R1ATC is regularly verified using PCR. R1ATC is used as an uninfected recipient strain in the trans-infection experiment into an usual host.

Experimental methods

Micro-injections. They were carried out as described in Santamaria (1987). Using a microcapillary needle (Femtotips, Boehringer) cytoplasm was drawn from infected embryos (R1A, NHA or R3A) and then injected into slightly dehydrated uninfected embryos (SimO or R1ATC). Isofemale lines were established after crossing the emerging females with uninfected males bearing the same genetic background (i.e. SimO or R1ATC).

Rearing conditions. Initially, injected lines were maintained as low density mass cultures in bottles at 25°C, on axenic medium (David, 1962). After 10 generations, of this regime, all strains were transferred to vials at 18° and 25°C, with two replicates per temperature. The lines were then maintained routinely by mass transfer for the rest of the experiment.

PCR/RELP. Total DNA was extracted from ovaries following the method of Kocher et al. (1989) or from whole individuals following the method of O'Neill et al. (1992). The *Wolbachia* 16S ribosomal subunit DNA sequence was amplified using specific primers 99F and 994R as previously described in O'Neill et al. (1992). PCR amplification products were subjected to V*sp*I digestion at 37°C for 3 h. The 16S rDNA sequence of *w*No presents a V*sp*I restriction site whereas the sequence of *w*Ha does not, which allows discrimination of the two strains by RFLP. PCR products of bi-infected flies exhibit a three-band pattern (Poinsot et al., 2000).

CI measurements. Tests were performed at 25°C. Fifteen 4 or 5-day-old virgin females were allowed to mate for 8 h with 25 virgin 3-day-old males in a bottle containing fresh axenic medium. Flies were then transferred to a box for oviposition. The aperture was fitted with a small Petri dish containing standard axenic medium darkened with vegetable charcoal powder. After 24 h, the adults were discarded and the eggs kept at 25°C for at least another 24 h duration before the hatching rate was estimated, generally from 200 eggs per line.

Level of cytoplasmic incompatibility. We used CIcorr, a corrected index of CI. The aim was to minimise the background noise due to the natural mortality of the cross (that is not related with CI). This background mortality was estimated from the mortality induced by males from injected but uninfected isofemale lines (i.e. lines where the bacteria had failed to be transferred) in crosses with uninfected SimO females. Failure of the infection transfer was defined as the absence of a positive PCR signal in the isofemale line in G3. Since males from these lines are not infected, the cross with SimO females is compatible and any mortality is background mortality (BgM.).

CIcorr is then defined as the percentage of eggs that do not hatch among those that would have survived in the absence of CI.

$$CIcorr(\%) = 100 \times \frac{(\%Unhatched - \%BgM.)}{(100 - \%BgM.)}$$

where %Unhatched is the percentage of unhatched eggs observed in the incompatible cross. CIcorr was set at 0 in the very rare cases where %Unhatched was lower than %BgM.

Statistical analysis. The trans-infection rate was analysed as a function of the donor strain, the recipient strain and their interaction using the partition of χ^2 test (Maxwell, 1961). This test provides, for discrete variables, a statistical analysis equivalent to an ANOVA (Winer, 1971). The same test was carried out to analyse the loss of the infection depending on the rearing temperature and the recipient strain. The level of CI induced by trans-infected lines was tested by an ANOVA [GLM procedure type IIISS of SAS (1989)], after arcsine transformation, as a function of the infection status, the recipient strain and their interaction.

RESULTS

Success rate of the trans-infection

The results, based on PCR + RFLPs carried out in G3, are presented in Table 1. The partition of χ^2 reveals that only the type of donor effect is significant. This is due to a higher rate of trans-infection when the donor strain is bi-infected. Indeed, the success rate is not significantly different between the two mono-infected donors (χ^2= 1.380, 1df, ns), at least when assessed in G3. However, the success rate obtained with the bi-infected donor strain is significantly superior to the average success rate of the two mono-infected donors (47.17% vs 24.24%; χ^2 = 6.842; 1df, P < 0.01). The absence of a recipient

TABLE 1. Success rate of the transinfection as a function of the recipient and the donor strains, followed by a partition of χ^2 test. In the case of bi-infected donor strains, the type of infection found in the recipient isofemale lines after a successful transinfection is indicated (no individuals mono-infected by *w*No were recovered). All PCR + RFLPs were carried out in G3 after transinfection. ns: not significant.

Recipient strain	Donor strain	n lines	% infected	(*w*Ha+*w*No)	(*w*Ha)
SimO	R1A(*w*Ha+*w*No)	19	52.63	4	6
	NHa(*w*Ha)	16	31.25		
	R3A(*w*No)	22	13.64		
	All donors	57	31.58		
R1A-TC	R1A(*w*Ha+*w*No)	34	44.12	11	4
	NHa(*w*Ha)	8	37.5		
	R3A(*w*No)	20	25		
	All donors	62	37.1		
All recipients	R1A(*w*Ha+*w*No)	53	47.17	15	10
	NHa(*w*Ha)	34	33.33		
	R3A(*w*No)	42	19.05		
	All donors	119	34.45		

Partition of χ^2 test

Effect	χ^2	d.f.	P
Donor	8.22	2	P < 0.05
Recipient	0.4	1	ns
D × R	0.68	2	ns
Total	9.3	5	ns

effect shows that the infection is established as easily in the "naive" strain (SimO) as in its usual host (R1A genetic background). We also found that mono-infected as well as bi-infected isofemale trans-infected lines could be recovered when a bi-infected strain was used as a donor. In our experiments, all mono-infected lines recovered in those conditions harboured *w*Ha. The relative proportion of mono and bi-infected lines recovered did not differ as a function of the recipient strain (χ^2 = 2.778, 1df, ns).

Cytoplasmic incompatibility

Before carrying out the injection experiment, we measured the CI level induced by the three donor strains. The percentage of unhatched eggs (estimated on 300 eggs per cross) were 71.3% (R1A); 57.7% (NHa) and 60.7% (R3A). Contrary to previous repeated observations (Merçot et al., 1995; Merçot & Poinsot, 1998), the *w*Ha variant (NHa strain) did not induce a significantly higher CI than the *w*No variant (R3A strain) (χ^2 = 0.581, 1df, ns). This might reflect a low level of infection of our NHa strain at the time of our injection experiment. Indeed, it has been found that, for a given *Wolbachia*, the level of CI is correlated with bacterial load in males (Breeuwer & Werren, 1990; Boyle et al., 1993; Bressac & Rousset, 1993; Solignac et al., 1994; Merçot et al., 1995).

Three generations after injection, we tested the level of CI induced by trans-infected lines. The results are shown in Table 2 and have been analysed using an ANOVA (Table 3). The recipient strain effect is significant (P < 0.05) as well as the infection status effect (P < 0.01). The interaction is not significant. The recipient strain effect is due to a higher CIcorr in trans-infected males of the "naive" SimO strain compared to males of the usual host (R1A genetic background) (67.2 ± 7.4% vs 37.3 ± 6.2%). Concerning the infection status, we compared the means of each infection status (LSMEANS/TDIFF statement in SAS 1989). This comparison shows that CIcorr was significantly higher in bi-infected males than in males mono-infected by *w*Ha (77.5 ± 7.1% vs 43.4 ± 7.4%, T = 3.31, P < 0.01) or by *w*No (77.5 ± 7.1% vs 35.9 ± 8.3%, T = 2.31, P < 0.05). There was no significant difference between *w*Ha and *w*No-infected males (T = 0.19, n.s). This latter result is in agreement with the absence of significant difference in CI levels between NHa and R3A donor strains before the trans-infection experiment began. Nevertheless, we found the usual significant difference in favour of *w*Ha when some of the trans-infected lines presented here were assessed again after a longer period (over 40 generations) in the laboratory. The percentage of unhatched eggs (estimated on 200 eggs per cross) were 88.5% (R1A*w*Ha6 line) and 67.0% (R1A*w*No17 line) (χ^2 = 26.72, 1df, P < 0.001).

Loss of the infection

The lines where trans-infection had been successful (as judged by a positive PCR in G3) were assessed by PCR + RFLP a second time after 18–20 months of routine maintenance in vials in the laboratory. During that time, two different temperature regimes had been used (18°C, i.e. about 20 generations, or 25°C, i.e. about 40 generations) with two replicates per temperature. Due to losses during this long period of storage, only one of the original two replicates per temperature regime was available for several lines at the time of our analysis. The proportion of cases did not differ significantly between SimO and R1ATC trans-infected lines both at 18°C (46.7% vs 58.8% respectively, χ^2 = 0.182, 1df, ns with Yates correction) and at 25°C (26.7% vs 15.0%, χ^2 = 0.470, 1df, ns). In order to keep as much information as possible, we calculated the proportion of replicates that were still infected (instead of the proportion of lines). The results are presented in Table 4. The partition of χ^2 reveals a significant effect of temperature. The infection was nearly always lost in replicates maintained at 18°C (46 times out of 49) while it was lost in slightly more than half of the repli-

TABLE 2. Cytoplasmic incompatibility in G3. See Materials and Methods for the calculation of the CIcorr index. se: standard error.

Recipient strain	Infection status	n lines	n eggs	% unhatched eggs ± se	% CIcorr ± se
SimO	*w*Ha+*w*No	6	1,200	84.1±9.2	80.7±11.1
	*w*Ha	9	1,700	66.1±9.4	58.9±11.4
	*w*No	2	400	70.0±0.0	63.6±0.0
	Uninfected	39	7,549	17.5±1.5	
R1ATC	*w*Ha+*w*No	4	785	75.6±8.0	72.6±9.0
	*w*Ha	10	1,590	44.5±6.7	29.4±7.6
	*w*No	5	1,000	33.2±5.4	24.9±6.1
	Uninfected	28	5,423	11.1±1.0	

TABLE 3. ANOVA on the level of cytoplasmatic incompatibility (CIcorr) in G3.

Source of variation	df	Mean square	F	P
Infection status	2	0.66	5.77	$P < 0.01$
Recipient strain	1	0.61	5.31	$P < 0.05$
Interaction	2	0.03	0.27	ns
Error	30	0.11		

cates maintained at 25°C (36 times out of 64) (93.9% vs 56.3%, $\chi^2 = 19.737$, 1df, $P < 0.001$). At 18°C, infection loss is similar in the two recipient strains (SimO: 96.2% vs R1ATC: 91.3%; $\chi^2 = 0.144$, 1df, ns). In contrast, at 25°C infection loss is highly significantly lower in the "usual" recipient strain (R1A genetic background) than in the "naive" SimO recipient strain (34.2% vs 88.5%; $\chi^2 = 22.823$, 1df, $P < 0.001$.).

DISCUSSION

Day-to-day *Wolbachia* transmission is believed to be strictly vertical maternal transmission. Yet, phylogenetic evidence reveals that horizontal transmission has occurred several times in the past, even between very distant taxons (Werren et al., 1995b; Zhou et al., 1998), thus demonstrating that *Wolbachia* can colonise new host species. The success of the establishment will then depend on the capability of the *Wolbachia* to grow in the new host, to induce CI (if we consider only this phenotype) and to be efficiently transmitted maternally. Here, we have tried to mimic this colonisation process of an uninfected host within the species *D. simulans*, using a microinjection technique and two recipient strains. One strain, SimO, is uninfected in the wild while R1ATC is the usual host of both *w*Ha and *w*No *Wolbachia* variants.

Success rate of the trans-infection

We have been able to transfer in both recipient strains each of the three possible infection types, i.e. *w*Ha mono-infection, *w*No mono-infection, as well as the *w*Ha + *w*No bi-infection. The rate of successful trans-infection among the isofemale lines obtained (41/119) was comparable to those reported in similar earlier experiments involving *Drosophila* species where the infection status of injected lines had been assessed in the very first generations after the injection (Rousset & de Stordeur, 1994: 6/12, Giordano et al., 1995: 9/24, Poinsot et al., 1998: 17/33). The rate of success of the trans-infection was superior when using bi-infected donor eggs. This might result either from a greater infection of bi-infected females or from a synergistic effect between the *w*Ha and *w*No variants. On the other hand, the rate of successful transfer did not differ significantly between naive and usual host, suggesting that the "naive" status did neither impede nor facilitate the initial establishment of the infection, at least in the conditions of our experiment. This finding is counter-intuitive (one might expect a more efficient transfer in the usual host). However, microinjection in embryos might not be representative of the (as yet unknown) routes followed by horizontal transmission in the wild. For example it has been demonstrated that *Wolbachia* can also be experimentally transmitted by injection in nymphs of *Trichogramma* (Trichogrammatidae) wasps (Grenier et al., 1998) or in the adult in terrestrial isopods (Juchault et al., 1994). In the case of the woodlouse *Armadillidium vulgare* (Latr.), (Oniscidae), wound-to-wound contact has been shown to be sufficient to induce transmission, since *Wolbachia* are present in hematocytes (Rigaud & Juchault, 1995). This suggests that inter-species natural transmission takes more indirect (and therefore more difficult) routes than injection into an egg. By injecting *Wolbachia* directly into the embryo at the most favourable stage (before pole cells formation), our protocol might have facilitated the installation of the bacteria to the point where potential differences between naive or usual hosts were not important for the success of the transfer.

TABLE 4. Infection loss in trans-infected isofemale lines after 18–20 months in the laboratory.

Recipient strain	Infection status in G3	Infection status in replicates kept at 18°C		Infection status in replicates kept at 25°C	
		n infected	n uninfected	n infected	n uninfected
SimO	*w*Ha+*w*No	0	10	1 (*w*Ha+*w*No) 1 (*w*No)	5
	*w*Ha	0	11	0	14
	*w*No	1	4	1	4
	Total	**1**	**25**	**3**	**23**
R1A-TC	*w*Ha+*w*No	1 (*w*No)	1	5 (*w*Ha+*w*No)	1
	*w*Ha	0	14	15	10
	*w*No	1	6	5	2
	Total	**2**	**21**	**25**	**13**

Cytoplasmic incompatibility

The most interesting finding is that CI was significantly higher in the naive host than in the usual host, regardless of the infection type (i.e. *w*Ha, *w*No or *w*Ha + *w*No). In a previous experiment, Rousset & de Stordeur (1994) trans-infected a naturally uninfected *D. simulans* strain (Watsonville, USA) with *w*Ha and *w*No. Seven generations post injection, they found that naive trans-infected males caused a weaker CI than males from the infected donor strain. However, due to the absence of an injection control (injection into usual hosts from a strain cured from its *Wolbachia* beforehand) it even was not possible to unambiguously attribute the low CI obtained to the naive genome (Watsonville) rather than to the injection procedure itself. Other results involving inter-species transfers (Boyle et al., 1993; Braig et al., 1994) show that when the infection is in the first few generations following a trans-infection, the level of CI can be significantly weaker than what it becomes after several generations or rearing in good conditions. Our own present results suggest the same idea (CI levels rising from 30–40% in G3 post-

infection to over 80% more than 40 generations later in our mono-infected trans-infected lines).

Loss of the infection

When checking the infection status of trans-infected lines after 18–20 months of routine maintenance at 25°C (i.e. about 40 generations) we found that infection loss had occurred in most naive trans-infected lines, but in only a third of usual host trans-infected lines, the difference being highly significant ($P < 0.001$). The loss of the infection can have two causes: (i) poor maternal transmission, (ii) lower viability of infected eggs (or individuals), especially in a situation of crowding. Our protocol does not allow to determine if only one or both factors played a role. We can however conclude that in temperature conditions (25°C) which are optimal for the rearing of the host, *Wolbachia* maintenance was significantly easier in the usual host than in the naive host. This difference was not apparent in more hostile conditions, i.e. at 18°C, a temperature found previously to be sub-optimal for the maintenance of the infection (Merçot & Poinsot, 1998). At this lower temperature, nearly all lines lost the infection regardless of the type of hosts (naive or usual), even though a period of 18–20 months at this temperature represents only about 20 generations of hosts (as compared to about 40 generations at 25°C). The rapid loss of a new infection has been found in other artificial transfer experiments. Van Meer & Stouthamer (1999) injected the *w*Ri *Wolbachia* variant from *D. simulans* to the microhymenopteran *Muscidifurax uniraptor* Kogan & Legner (Pteromalidae). The initial transfer was successful but maternal transmission was poor and the infection was lost in 6 generations despite optimal conditions of rearing for the host. In Isopods, Rigaud & Juchault (1995) found that in inter-species trans-injections, the initial transfer was often successful but that in most cases the bacteria were not transmitted to the offspring at all. Working with the same donor host (the woodlouse *Armadillidium vulgare*), Bouchon et al. (1998) found that in naive host species the infection could have lethal pathogenic effects, which was never the case in the usual host. On the other hand, Braig et al. (1994) obtained a stable infection after transferring the *Wolbachia* of the mosquito *Aedes albopictus* Skuse (Culicidae) into *D. simulans*, demonstrating experimentally that *Wolbachia* can cross species barriers over relatively large phylogenetic distances.

Conclusion

Our results show that when transferred into a new host, both *w*Ha and *w*No cause more CI if the host is naive than when it is not. We also found that these *Wolbachia* seem less able to maintain a durable association with the naive host. As previously stated, our protocol does not allow us to decide if this is due to poor transmission and drift alone or if a slightly deleterious effect linked to the infection played a role. In any case, our results are in full agreement with the data and the ideas presented by Clancy & Hoffmann (1997), based in part on the model of host and *Wolbachia* coevolution developed by Turelli (1994). These authors conclude that CI will tend to be high in a new host (following horizontal transfer), when the host has not had time to evolve any resistance. Also, at this early stage, the bacteria are not yet fully adapted to the new host and are not expected to achieve a perfect maternal transmission, nor to be completely harmless for the female host, in particular regarding fecundity. Over time, the situation is expected to evolve on all fronts. First, any gene diminishing the effects of CI will be selected for in the host, and CI is expected to diminish with time. Indeed, CI is low and sometimes undetectable in *Drosophila melanogaster* Meigen (Drosophilidae), and totally absent in the *w*Au infection of *D. simulans*, both infections believed to be very ancient (Solignac et al., 1994; Clancy & Hoffmann, 1997). Second, *Wolbachia* obviously bears a strong selective pressure to improve its own transmission, and to a certain extent the female host will bear the same pressure (because infected eggs are protected from CI). Therefore, maternal transmission is expected to be highly efficient in old infections, especially if the CI capability has been lost and can not compensate for a poor transmission any more. Finally, both the bacteria and the female host are selected to maximise female fecundity, so an old infection is not expected to reduce fecundity. Again, it seems exactly to be the case in the *w*Au infection of *D. simulans*, where transmission is probably nearly perfect in the field and where CI and deleterious effects on the female are not detectable (Clancy & Hoffmann, 1997). Such an old *Wolbachia* infection is however expected to slowly drift into extinction because, without CI, even a near perfect transmission is not perfect enough. Alternatively, this peaceful decline can be abruptly shortened by the sudden invasion of a new high-CI variant following horizontal transfer or migration. A case in point might be the spectacular spread of the *w*Ri variant taking place right now in *D. simulans* populations, some of which probably harboured already the "harmless" (and apparently doomed) *w*Au variant (Turelli & Hoffmann, 1995).

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