

polished my prose. This is paper 99–114 of the Institut des Sciences de l'Evolution.

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Wolbachia as a speciation agent

Bacteria of the genus *Wolbachia* are reproductive parasites of arthropods. They are cytoplasmically inherited (i.e. from mothers to daughters, like mitochondria) and their phenotypic effects on their hosts range from induction of parthenogenesis in certain hymenopteran groups, to feminization of genetic males in isopod crustaceans and to induction of cytoplasmic incompatibility in many insects¹ (Box 1). Researchers have envisioned *Wolbachia* playing a role in insect speciation by generating reproductive isolation^{2–4}. However, theory predicts that *Wolbachia* might not have a major role in the process of speciation⁵. A recent paper by Shoemaker *et al.*⁶ demonstrates experimentally how *Wolbachia* might act as a speciation enhancer, thus bridging the gap between theoretical predictions and experimental results.

But how can *Wolbachia* drive speciation? Three ways have been suggested⁴. The first suggestion is induction of parthenogenesis (assuming that the infection becomes fixed in the species). This process appears to occur in the hymenopteran *Encarsia formosa*⁷; males in this species can be obtained when mothers are treated with antibiotics,

however, they are incapable of mating. The second way is offered by bidirectional incompatibility (Box 1). If a population is infected with two different strains of *Wolbachia* that are incompatible with each other, then bidirectional incompatibility will act as a post-zygotic reproductive barrier. A promising candidate is the species complex of the wasp genus *Nasonia*^{2,3}. The third way is offered by unidirectional incompatibility (Box 1). In this case, *Wolbachia* is acting as just one of the reproductive barriers enhancing speciation between two taxa. It is in this area of *Wolbachia* research that Shoemaker *et al.*⁶ offer a new perspective, providing the first experimental data.

The work focuses on two closely related species of *Drosophila*, *D. recens* and *D. subquinaria*, which belong to the *subquinaria* species group. Both are ecologically similar, live in the cooler regions of North America, and use mushrooms as mating and ovipositing sites. *D. recens* is distributed across the northeastern USA and southeastern Canada, whereas *D. subquinaria* spreads across the northwestern USA and southwestern Canada. They live in sympatry over a broad area in central Northern USA. The

principal finding is that *Wolbachia*-induced unidirectional incompatibility, coupled with a behavioural isolation mechanism acting in the reciprocal direction, establishes a reproductive barrier between the two *Drosophila* species.

When Shoemaker *et al.*⁶ crossed *D. recens* and *D. subquinaria*, they revealed an asymmetrical behavioural isolation mechanism. Although *D. recens* females will not mate with *D. subquinaria* males (Table 1), *D. recens* males will mate with *D. subquinaria* females. In fact, there was no significant difference between the latter interspecific cross and the pairing probabilities within either species.

In a screening for *Wolbachia* infection, *D. recens* was found to be positive, whereas *D. subquinaria* was not infected. To investigate the role of *Wolbachia* in crosses involving *D. recens*, the number of offspring obtained from matings between infected *D. recens* males and uninfected *D. subquinaria* females, was compared with the number of progeny obtained from matings between *D. recens* males that were treated with antibiotics (and thus *Wolbachia*-negative) and *D. subquinaria* females. The number of progeny in the first cross was significantly lower (Table 1), suggesting a *Wolbachia*-mediated barrier to this cross. No statistically significant difference in the number of offspring was observed for all the other control crosses.

However, a possible involvement of other post-zygotic factors restricting gene flow has not yet been discarded. Two post-zygotic factors are most important as reproductive barriers, namely hybrid sterility and hybrid breakdown. Hybrid sterility was tested by crossing hybrid males or females with either of the parental species. The results suggested that hybrid males were invariably sterile, whereas hybrid females were fertile (Table 1). This is in agreement with Haldane's rule⁸ (i.e. if a partial reproductive barrier exists this should affect the heterogametic sex most severely) and suggests the presence of an incomplete post-zygotic isolation barrier.

Hybrid breakdown is the inviability or sterility of progeny resulting from a backcross of hybrid progeny with either of the

Box 1. Cytoplasmic incompatibility: definitions and theoretical predictions

Cytoplasmic incompatibility: can be defined as the failure of a cross to produce any offspring (or only male offspring in haplodiploid species) because of cytoplasmic factors. It can either be unidirectional or bidirectional³.

Unidirectional incompatibility: the cross between a *Wolbachia*-infected male and an uninfected female fails to produce any progeny whereas the reciprocal cross (uninfected male X infected female) is normal. Theory suggests that natural selection (on the parasites) acts on increasing the fecundity of the infected females^{5,9}, rather than increasing the incompatibility levels between infected versus uninfected. The consequence is that strains with lower incompatibility levels, but with a lower cost in the fecundity of the host, might be selected for.

Bidirectional incompatibility: the cross between two individuals infected with different strains of *Wolbachia* fails to produce any progeny. Theory predicts that natural selection (on the parasites) will act towards establishing a single infection in populations that are infected with two different *Wolbachia* strains, if sufficient gene flow exists between the two populations⁵. In the example discussed in the text (*Nasonia* species complex), it is unclear whether infection with different *Wolbachia* strains preceded, and thus drove, speciation or followed species formation.

parental species. Hybrid progeny have a set of chromosomes from each parental species. Progeny resulting from a cross of one of these hybrids with either of the parental species do not, and thus a 'hybrid breakdown' might occur leading to sterility and/or inviability. Backcross hybrids were mated with either of the parental species to test whether there was any hybrid breakdown effect. Most of the male backcross hybrids were sterile – such as the F₁ male hybrids – whereas the female backcross hybrids did not show any significant reduction in the number of progeny they produced, thus suggesting an absence of 'breakdown' effects (Table 1).

Shoemaker *et al.*⁶ suggest that this might be one of the ways *Wolbachia* acts as a speciation agent. Coupled with one pre-zygotic barrier (unidirectional behavioural isolation) and another post-zygotic barrier (hybrid male sterility), *Wolbachia* acts as a reproductive barrier (and, thus, a speciation inducer) between the two species. This is the first explicit demonstration of how a *Wolbachia* infection, causing unidirectional incompatibility, contributes to the establishment of a reproductive barrier between the two species.

Although theory suggests that *Wolbachia* parasites themselves might not be important as speciation agents, these data suggest a possible role for *Wolbachia* as one of the many reproductive barriers between two incipient species. Therefore, rather than envisioning *Wolbachia* as the sole driver of the whole speciation process, Shoemaker *et al.*⁶ suggest that *Wolbachia* can act as one of the speciation agents. One particular role for *Wolbachia* might be in reducing gene flow between geographically separated, genetically divergent populations, which subsequently overlap before reproductive barriers are complete. The data offered by Shoemaker *et al.*⁶ suggest that *Wolbachia* might be a reinforcement mechanism in such situations.

Some comments should be made on the sampling strategy used by Shoemaker *et al.*⁶, which perhaps reduces the force of the paper's arguments. Species samples were not taken from places where the two species occur in sympatry, but from sites on opposite sides of the USA. Therefore, we do not actually know whether behavioural isolation and hybrid sterility occur in the zone of sympatry with the same intensity as in the tests described for (highly) allopatric populations. Given that the behavioural isolation mechanism is not perfect (some matings do occur), it is at least possible that the frequency of matings, in the cross in which behavioural isolation is

Table 1. Synopsis of the most important crosses performed

Cross no.	Male	Female	Observation tested	Explanation of observation
1	<i>subquinaria</i>	<i>recens</i>	Mating frequency	Behavioural isolation
2	<i>recens</i> ^W	<i>subquinaria</i> ⁰	Offspring production	Cytoplasmic incompatibility
3	F ₁ hybrid	Either parental species	Offspring production	Hybrid male sterility (Haldane's rule)
4	Either parental species	F ₁ hybrid	Offspring production	No female sterility effect
5	Either parental species	Backcross or F ₂ hybrid (offspring of cross no. 4)	Offspring production	No hybrid breakdown effect

Key: *recens*^W = *Wolbachia*-infected *D. recens*; *subquinaria*⁰ = uninfected *D. subquinaria*. Details from Ref. 6.

observed, will decrease as we move from the allopatric zone to the sympatric zone of the two species. More careful sampling would have provided a stronger case for what the authors suggest.

Notwithstanding this argument, the first step has already been taken towards elucidating *Wolbachia*'s role (when the effect is unidirectional incompatibility) in speciation events. The right questions have been asked and the first evidence is certainly promising. It should not take long before new, and more complete, studies are published. *Wolbachia*'s share of the explanation of what Darwin called 'the mystery of the mysteries' has slowly started to emerge. We should certainly expect more wonders from this amazing reproductive parasite.

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