Rescuing *Wolbachia* have been overlooked...

*Wolbachia*, intracellular bacteria transmitted through the egg, have been estimated to infect more than 16% of all insect species, as well as other arthropods. They distort their hosts’ reproduction, inducing parthenogenesis, feminization and cytoplasmic incompatibility. This favours the reproduction of infected female hosts at the expense of uninfected females. Here we show that several *Wolbachia* strains that cannot generate modifications in host sperm can still rescue the modifications caused by other strains as long as the two strains are sufficiently closely related.

Cytoplasmic incompatibility causes embryonic mortality in crosses between infected male hosts and females that are either uninfected or infected with an unrelated *Wolbachia* strain. Cytological studies suggest that *Wolbachia* modifies the paternal chromosomes during spermatogenesis and this modification is rescued in eggs of females infected with the same strain of *Wolbachia* during fertilization.

In *Drosophila* two types of strains of *Wolbachia* have been described: mod− resc−, capable of modifying spermatozoa and rescuing this modification in eggs, and mod− resc+, incapable of either modifying sperm or rescuing the modification in eggs. It has been hard to explain the presence of mod− resc− strains in insect hosts as it is difficult to understand how these strains could spread into host populations without the action of cytoplasmic incompatibility.

Alternatively these mod− resc− strains might have failed to rescue cytoplasmic incompatibility in test crosses because of strain differences between the mod− and mod+ strains, as in cases of bidirectional incompatibility. Until recently there was no way to type *Wolbachia* strains independently of crossing the hosts carrying the strains. We have recently cloned and sequenced the gene (wosp) for a major surface protein of *Wolbachia* that shows variability between *Wolbachia* strains. The sequence of this gene from different *Wolbachia* strains infecting *Drosophila* shows that many of the crosses previously used to test the ability of mod− strains to rescue cytoplasmic incompatibility were between distantly related *Wolbachia* variants.

We have crossed females from previously described mod− resc− strains with males that, on the basis of wosp gene sequences, carry closely related mod+ resc+ *Wolbachia* strains (Fig. 1) and find that the mod− resc− strains can indeed rescue the sperm modification of these related *Wolbachia* strains. For example, the *Wolbachia* strains infecting *D. melanogaster* Canton-S and wosp(C23) are closely related by wosp sequences, and crosses between Canton-S females and wosp(C23) males are completely compatible (Fig. 2a). Similarly, the mod− strain of *Wolbachia* originally described in *D. mauritiana* and subsequently transferred to the uninfected *D. simulans* Watsonville strain did not express cytoplasmic incompatibility in crosses with either uninfected females or females of the *D. simulans* Riverside strain infected with a *Wolbachia* variant known to display high levels of cytoplasmic incompatibility.

Indeed, transfected Watsonville females fail to rescue the cytoplasmic incompatibility phenotype in crosses with Riverside males, but they were fully compatible in crosses with males of the R3A strain (Fig. 2a), which carries the mod− resc− 'Noumea' *Wolbachia* variant, a strain closely related by wosp gene sequences (Fig. 1). In similar crosses we found that the mod− *Wolbachia* strain described in *D. simulans* populations from Coffs Harbour, Australia, rescues the cytoplasmic incompatibility imprint of the *Wolbachia* mod+ resc+ variant infecting *D. melanogaster* after transfer into *D. simulans* (unpublished data).

These strains, which fail to modify sperm but can rescue the modification in eggs, might do so because they show differential affinities for male and female germline tissues, being more abundant in eggs and less abundant in testes. We tested this hypothesis with dot blots of nucleic acids extracted from testes and ovaries, with the *Wolbachia dnaA* gene as a probe (Fig. 2b) and by western blots of protein extracts from the same tissues with a polyclonal anti-WOSP antiserum (data not shown).

All mod− resc+ and mod− resc− *Wolbachia* strains infected the testes and ovaries of their respective hosts, infecting ovaries at higher levels than testes, but there was no simple correlation between the absolute densities of *Wolbachia* in testes and the ability of these strains to act as either a mod− or a mod+ strain. With at least one of these strains the mod− phenotype is independent of the host genome, so mod− *Wolbachia* variants might be genetically incapable of imprinting *Drosophila* sperm. Thus the molecular mechanisms of sperm...
modification and rescue might involve multiple Wolbachia genes.

The existence of Wolbachia strains that do not modify sperm but can rescue the cytoplasmic incompatibility phenotype has been predicted by theory\(^1\)\(^2\)\(^1\)\(^4\). Our work suggests that previously classified mod ‘resc’ strains of Wolbachia can in fact rescue cytoplasmic incompatibility and can therefore be considered mod ‘resc’\(^+\). This finding helps explain how Wolbachia mod \(^-\) strains could have spread into insect populations without the action of cytoplasmic incompatibility. Wolbachia strains that can rescue but not cause cytoplasmic incompatibility can be expected to increase in frequency in populations by essentially parasitizing the cytoplasmic incompatibility sperm modification effect of a related Wolbachia strain.

The potentially lower fitness costs and perfect maternal transmission of these strains\(^5\)\(^6\)\(^7\)\(^8\) might explain how they could displace the initial strain that they parasitized in order to invade the host population. This is in accordance with models predicting that Wolbachia will evolve towards neutrality with respect to cytoplasmic incompatibility as long as maternal transmission frequency can be increased\(^9\).


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