

# Fighting back against male-killers

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**Male-killing endosymbionts create a genetic black hole into which host nuclear genes vanish. In a recent paper, Hornett *et al.* transferred male-killing *Wolbachia* between different strains of the butterfly *Hypolimnas bolina* through hybridization and backcrossing. Their results provide unambiguous evidence of genetic variation for resistance to male-killers. A possible consequence of such variation is that male-killing might appear and disappear quickly on an evolutionary timescale.**

## Male-killing endosymbionts

Many insect species are infected by cytoplasmic endosymbionts that are transmitted through eggs from females to their offspring. Because this mode of transmission makes females the more valuable sex for the evolutionary success of maternally transmitted elements, many endosymbionts manipulate host reproduction in ways that increase the production of female offspring. Male-killing is one such strategy. Male-killers spread because the death of male embryos reduces the levels of competition, cannibalism or inbreeding experienced by the survivors, thus increasing the fitness of the sisters of the infected males that had been killed.

Numerous species of insects, including flies, beetles, butterflies, moths and wasps, are infected by male-killers belonging to a variety of bacterial groups [1]. Although male-killing associations have evolved repeatedly, they are a genetic disaster for the host species. Now, Hornett *et al.* [2] have shown that insects can evolve resistance to one type of male-killing bacterium, *Wolbachia*.

## The problem with male-killers

Because infection results in the death of male embryos, a male-killing endosymbiont that is transmitted to all the offspring of an infected female and that kills all of the males removes half of the nuclear genes carried by the infected members of a population every generation. In the following generation, the surviving nuclear genes, which have been passed on only to the females, are again split 1:1 into male and female offspring, with those going to males being lost, because of the continued transmission of the endosymbiont (Figure 1). Thus, no nuclear genes last for long within the infected component of the population. Because the infected females are maintained by backcrossing to uninfected males every generation, the infected component of the population is a genetic black hole, making essentially no long-term contribution to a population.

Hornett *et al.* [2] suggest that the impact of male-killers is even greater, as such endosymbionts entail a 'double fitness cost of mortality and failure to produce the rare sex.' The deficiency of males at the population level would appear to make them the more valuable sex, because they contribute more nuclear genes, on average, to the next generation than do females [3]. However, with 100% transmission of the endosymbiont and 100% killing of the infected males, the only nuclear genes that persist in the long term are those residing within the uninfected component of the population, where the sex ratio is 1:1, thus making males and females equally valuable. Under these conditions, there is no selective advantage to the production of excess males, despite their rarity at the population level. However, if maternal transmission of the endosymbiont is incomplete, there can be selection on autosomal genes in favor of a male-biased sex ratio [4].

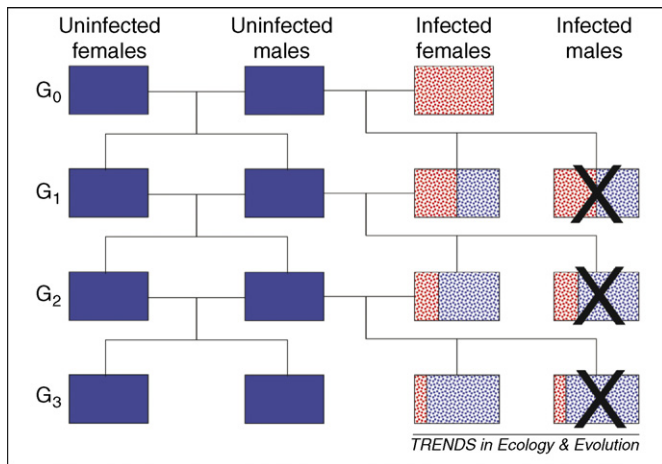
More importantly, suppose a dominant mutation arises that confers resistance to male-killing. Infected females carrying such a mutation would produce viable sons that also carry the mutation. Such males could mate with infected females and thereby rescue their genes from the genetic black hole. If the prevalence of infection is high, with an abundance of infected females, males carrying such a mutation would have exceptionally high mean fitness, leading to the rapid spread of the resistance allele, as well as of genes causing a male-biased sex ratio.

Despite the heavy toll taken by male-killers, there is little evidence for the evolution of resistance by their host species. In several species that have been examined, including *Drosophila bifasciata* [5,6], *Drosophila innubila* [7] and the butterfly *Acraea encedon* [8], there is little or no genetic variation for suppression of the male-killing effect of these endosymbionts. At least some of these endosymbiont infections are old, ranging from at least 100 years [9] to tens of thousands of years [6]. One interpretation of these negative results is that insects do not have the genetic wherewithal to evolve resistance to male-killing endosymbionts, making them an unsolvable evolutionary problem.

## Resistant butterflies

In a recent paper, Hornett *et al.* [2] show that the evolutionary problem of male-killing endosymbionts can be solved genetically. The nymphalid butterfly *Hypolimnas bolina* is geographically widespread, from Madagascar, through India and Southeast Asia, and across the islands of Oceania [10]. Several populations that have been examined are infected with the same strain of *Wolbachia*, designated *wBo11*. Infected butterflies from Southeast Asia, Borneo and the Philippines produce even sex ratios,

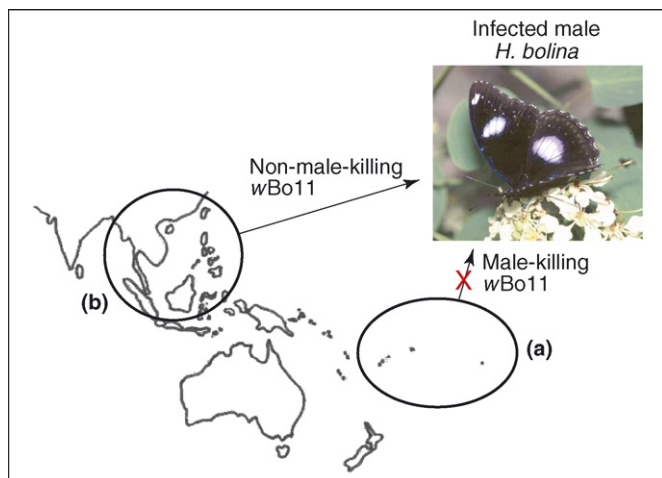
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**Figure 1.** Schematic diagram showing how the infected component of a species infected with a male-killing endosymbiont must be maintained by mating to uninfected males every generation. This results in the perpetual dilution of the nuclear genes in the infected component of the population. The nuclear genes present in the starting generation (G<sub>0</sub>) of the infected individuals are shown in red and those in the uninfected are in blue. *Wolbachia* infection is indicated by stippling. The same crossing scheme shows how the nuclear genes of one population (red) can be replaced by the nuclear genes of another population (blue), resulting in the introgression of *Wolbachia* from the red to the blue genetic background.

with no evidence of male-killing. By contrast, in the South Pacific islands of Fiji, Samoa and French Polynesia, *Wolbachia* infections cause male-killing, resulting in strongly female-biased populations [11].

Hornett *et al.* hybridized different strains of butterflies and then backcrossed the infected hybrid females to males of a given strain. In this way, they were able to move the *Wolbachia* via introgression from Moorea butterflies into those with a Southeast Asia genetic make-up, and, reciprocally, from Southeast Asia butterflies into those carrying Moorea genes. These reciprocal introgressions showed that male-killing is a function of the genetic background of butterflies: Moorea genes enabled male-killing, whereas those from Southeast Asia prevented it (Figure 2). Data



**Figure 2.** Distribution of the male-killing phenotype in *Hypolimnas bolina*. (a) Populations where *Wolbachia* strain wBo11 causes male killing include those in Fiji, Samoa and Moorea (French Polynesia). The X indicates that the production of infected males is prevented by *Wolbachia* in these populations. (b) *Wolbachia* infections in Malaysia, Vietnam, China, Borneo and the Philippines do not cause male killing and, thus, allow the production of infected males. Based on Refs [2,10]. Photograph reproduced with permission from Stephan Shuichi Haupt.

from segregation analysis are statistically consistent with a single dominant locus being responsible for the resistance to male killing.

Given the potentially huge selective advantage of resistance, why do some populations of *H. bolina* still suffer the male-killing effect of infection? One possibility, favored by Hornett *et al.*, is that the alleles conferring resistance have not yet spread to the susceptible populations. If this explanation is correct, then the Polynesian populations would rapidly lose male-killing once such alleles invade. This has the interesting consequence, noted by Hornett *et al.*, that male-killing infections might experience high 'extinction' rates, because their hosts evolve resistance. If so, then an equilibrium incidence of male-killing infections among species requires a high origination rate of male-killing to balance out the high rate of loss. Thus, on an evolutionary timescale, male killing might arise even more frequently than the phylogenetic analyses suggest. Perhaps, on these longer timescales, many insect lineages experience brief episodes of male-killing infection.

Male-killing infections might also be lost from a species before resistance evolves. The lack of phylogenetic congruence between *Wolbachia* strains and their insect hosts indicates that these infections are short lived on an evolutionary timescale, regardless of their phenotype [12–14]. It is also possible that species infected with male-killers experience higher rates of extinction if many females go unmated because of the shortage of males. They might also go extinct if such species harbor significantly reduced genetic variation, because most nuclear genes within a species are descended from uninfected individuals (Figure 1).

Finally, rather than being evolutionarily transient, it is possible that the geographical variation in resistance in *H. bolina* will not result in the spatial spread and invasion of alleles conferring resistance. For instance, if the populations of *H. bolina* from Southeast Asia and Polynesia are locally adapted, perhaps owing to mimetic polymorphism [9], this could reduce the rate of gene flow among populations.

### An alternative pathway to male-killer suppression

The study by Hornett *et al.* [2] demonstrates genetic variation for resistance to the male-killing effect of an endosymbiont. Another means by which resistance might evolve is via reduced transmission fidelity of the infection. In fact, two studies published 50 years ago found just this: Malogolowkin [15] found possible genetic variation in natural populations of *Drosophila willistoni* for the transmission fidelity of a male-killing *Spiroplasma* infection, although it was not known then that *Spiroplasma* was the responsible agent. Females of a *Spiroplasma*-infected strain were crossed and backcrossed to males of strains collected from several populations. Crosses to most strains resulted in all but complete male-killing. However, crosses to two out of three strains tested from northeastern Brazil (Recife) led to a high proportion of male offspring. Subsequent crosses of females from these lines never resulted in male-killing, regardless of the male type to which they were crossed. Although not suggested by Malogolowkin [15], this indicates that the *Spiroplasma* infection had been lost. Perhaps the occurrence of transmission

suppressors accounts for the low prevalence of infection of *D. willistoni* by *Spiroplasma* [16]. In the other study, Cavalcanti *et al.* [17] reported that *Drosophila prosaltans* is infected with an unidentified cytoplasmically transmitted male-killer, and that it is polymorphic for a recessive allele that prevents transmission of the male-killer.

### Conclusion

The bottom line from the studies by Hornett *et al.* [2], Malogolowkin [15] and Cavalcanti [17] is that infection of a species by male-killing endosymbionts might not be such a hopeless evolutionary situation after all. However, several questions remain. By what molecular or developmental mechanism do *Wolbachia* kill males, and how do genes conferring resistance prevent this? What is the level of polymorphism within populations for resistance to male-killing, and how does this correlate with infection prevalence? Finally, why have some species apparently failed to evolve resistance to long-established male-killing infections? Answers to these and other related questions will help us to further understand the complex and fascinating world of host–symbiont interactions.

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## Lateral thinking on data to identify climate impacts

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**It has never been more urgent to identify the potential impacts of climate change. In our quest for information, we often rely on records that reveal how organisms and systems responded to past climates. A new study by Miller-Rushing *et al.* uses some unorthodox archive material (photographs and herbarium specimens) to examine changes in flowering phenology in the USA. Their approach suggests that we have failed to think-outside-the-box and have been overlooking a valuable resource for climate-impact research.**

### Climate change impacts

It has been suggested that we are approaching a point of no return, beyond which climate change might be irreversible [1]. We need to be forewarned to be forearmed, and we need to understand and predict what the consequences will be for life on Earth. We need this information to identify where problems might arise not only for the conservation of species, but also for the sustainable use and protection of natural resources and crop protection and production. Recent reported changes in the phenology of plants and animals [2] demonstrate a clear response to temperature across large geographical areas, such as Europe and North America. This is important for two reasons; first, it demonstrates to the public and policymakers that change is already happening, even with the relatively modest

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