Male-Killing Bacteria in Insects: Mechanisms, Incidence, and Implications

Gregory D.D. Hurst and Francis M. Jiggins
University College London, United Kingdom

Bacteria that are vertically transmitted through female hosts and kill male hosts that inherit them were first recorded in insects during the 1950s. Recent studies have shown these "male-killers" to be diverse and have led to a reappraisal of the biology of many groups of bacteria. *Rickettsia*, for instance, have been regarded as human pathogens transmitted by arthropods. The finding of a male-killing *Rickettsia* obligately associated with an insect suggests that the genus' members may be primarily associated with arthropods and are only sometimes pathogens of vertebrates. We examined both how killing of male hosts affects the dynamics of inherited bacteria and how male-killing bacteria affect their host populations. Finally, we assessed the potential use of these microorganisms in the control of insect populations.

Female insects commonly interact with bacteria they pass on to their progeny. These inherited bacteria are often beneficial symbionts that play a key role in host metabolism. In many cases (e.g., the aphid symbiont *Buchnera*), the bacteria are maintained in a special host organ, the bacteriome, with the host controlling transmission to progeny, and show evidence of cospeciation (1,2). In these cases, destroying the bacteria (e.g., through antibiotic treatment) causes a profound loss of host performance. In other cases, inherited bacteria are not integrated into host physiology and anatomy and do not show long-lived relationships with their host, as indicated by a lack of cospeciation (3). These bacteria may be broadly separated into two classes. First, bacteria maintained through a phase of horizontal transmission (e.g., *Rickettsia prowazekii*), with transmission to other arthropod hosts often occurring through a vertebrate or plant intermediate host (infection of the intermediate host and new acquisition of infection follow from host feeding); second, bacteria that rarely show horizontal transmission, but are maintained because they manipulate host reproduction. One set of manipulations manifested by these bacteria is increasing

investment in daughters at the expense of sons. In these cases, particular host lines produce female-biased sex ratios, a trait that is inherited but curable with antibiotics. We considered one class of these, the male-killing bacteria, in which infection of a female results in the production of female-biased broods because male progeny die during embryogenesis.

Systematics of Male-Killing Bacteria

Molecular systematic approaches have shown that male-killing bacteria derive from many different clades. In most cases, the data come from DNA sequencing of bacteria associated with the trait and confirmation of the trait association by polymerase chain reaction across infected and uninfected lines. Because inherited microorganisms are difficult to culture, Koch's postulates have been fulfilled formally in only two cases (4,5). Given this caveat, male-killing bacteria have been found within the genus *Spiroplasma* (Mollicutes) (4,6), the Flavobacteria-Bacteroides group (7), and the gamma and alpha subdivisions of the proteobacteria (5,8,9) (Figure).

Male-killing bacteria derive from arthropod-associated bacterial clades that are not themselves male-killers. The clades can be separated into two types according to the transmission mechanisms of bacteria within them: first, entirely horizontal transmission or a mix of horizontal and vertical transmission; and second,
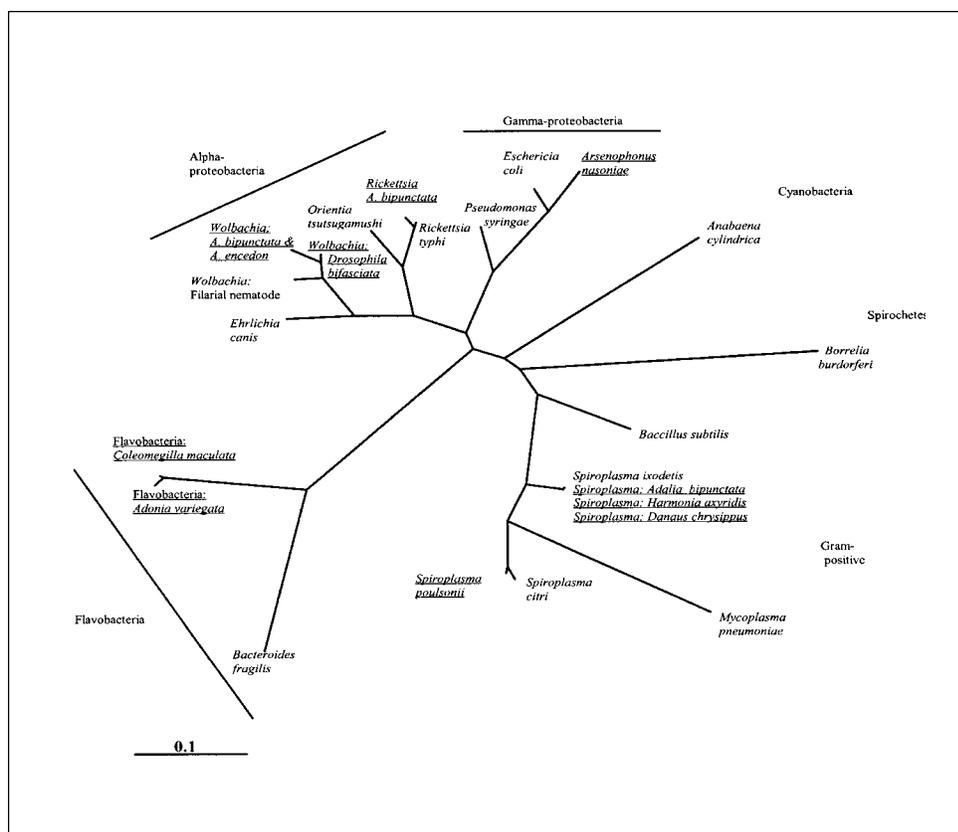
Perspectives

Figure. Phylogenetic relationship of male-killers and a selection of other eubacteria inferred from 16S rDNA sequences, using maximum likelihood implemented on PAUP*. The male-killing bacteria (underlined) have been labeled with the name of their insect host if a species name is not available. The relationships of the major bacterial groups are uncertain.

horizontal transmission that is not epidemiologically important. In the first type of clade are the genera *Spiroplasma* and *Rickettsia*. In *Spiroplasma*, most members have either horizontal transmission only (after feeding on a plant host) or a mix of horizontal and vertical transmission between arthropod hosts (10). *Rickettsia* most commonly have a mix of horizontal and vertical transmission, with horizontal transmission occurring after feeding on a vertebrate host. As recently as 10 years ago, *Rickettsia* was regarded as one of the vertebrate pathogens borne by arthropods. However, *Rickettsia* that show transmission after feeding on plant hosts are increasingly being recognized (11), and the finding of a male-killing *Rickettsia* in ladybird beetles (8) suggests that the group is associated with arthropods, some members of which cause disease in vertebrates. Other male-killing strains of *Rickettsia* will most likely be found. However, whether a bacterium from these groups could evolve male-killing yet retain horizontal transmission between females via feeding on a plant or vertebrate host has not been

established. The fact that male-killers derive from such groups suggests this possibility.

In the second type of clade, vertical transmission rates far exceed those of horizontal transmission. *Wolbachia* and the flavobacterial lineage associated with arthropods are in this group. *Wolbachia* are usually maintained through manipulation of their host's reproduction (12). The closest relative of the flavobacterial male-killer is *Blattabacterium*, the beneficial inherited bacterium of cockroaches and termites (13).

Male-killing, a trait that evolves in bacteria already maternally inherited in arthropods, can occur if the ancestral agent is obligately vertically transmitted or a mix of vertical and horizontal transmission is present. Therefore, male-killing strains are likely to be common in the genus *Spiroplasma* and the alpha group of proteobacteria. Furthermore, the diversity of agents suggests that there is no taxonomic bar to where the transition to male-killing can take place. Thus male-killing strains are also likely to be found in the spirochetes and perhaps the beta

and delta divisions of the proteobacteria, as these groups are known to be vertically transmitted within arthropods.

Although vertical transmission of male-killing bacteria is the rule, transmission between host species has occurred. In *Spiroplasma*, the relatives of *S. ixodetis* cause male-killing in distantly related hosts (a butterfly and a ladybird beetle) (6,14). The evolutionary distance between beetles and butterflies indicates that the bacteria do cross between host species over evolutionary time.

Host Species Affected

The incidence of male-killing bacteria varies with host ecology and biology. The trait of male-killing is adaptive when the death of males promotes the survival of female siblings. If the bacteria can be transmitted only vertically, the death of male hosts can at worst be neutral (i.e., they cannot transmit the bacterium). Death of males is adaptive if it increases the survival of sibling females, who bear the same bacterium by virtue of common descent.

The features of host biology and ecology that increase the benefit to the bacterium of killing male embryos are sibling egg consumption (females eat their dead brothers), antagonistic interactions between siblings (male-killing may reduce both cannibalism of females and the intensity of competition between siblings), and deleterious inbreeding (15-17). These observations explain why male-killer hosts commonly lay eggs in clutches. Incidence is highest where there

is also sibling egg consumption, as with coccinellid (ladybird) beetles. Approximately half of aphidophagous species bear male-killers, and one species (*Adalia bipunctata*) is host to at least three male-killing bacteria (6,8,9).

Male-killing bacteria have been recorded only in insects. However, the range of insect hosts is wide, with a variety of different sex determination systems. Given that close relatives of male-killing bacteria are found in noninsect arthropods (e.g., *Spiroplasma* and *Rickettsia* in ticks) and the conditions for the spread of male-killing strains are met outside insect hosts, cases of male-killing are likely to occur in species other than insects. Two examples merit particular examination. First, infection with *Orientia tsutsugamushi* is associated with production of all-female broods in the trombiculid mite, *Leptotrombidium fletcheri* (18,19); in this example, the nature of the resultant sex-ratio distortion (primary vs. secondary bias) needs to be assessed. Second, in the case of *Spiroplasma ixodetis* and its tick host *Ixodes pacificus*, the association of closely related bacteria with male-killing in insects needs to be assessed.

Prevalence of Male-Killers in Natural Populations

The prevalence of male-killers in natural populations varies with host species (Table 1). A prevalence value of 5%-50% might be "normal" among female hosts; however, in some cases prevalence is very low (e.g., 1% in *Drosophila*

Table 1. Prevalence of male-killers in natural populations of their insect hosts (proportion of females infected)

Bacterium	Host	Prevalence (%)	Ref.
Spiroplasma sp.	*Adalia bipunctata*	0-22	6
(*S. ixodetis* relative)	*Harmonia axyridis*	0-49	35
	Danaus chrysippus	40	14
Spiroplasma poulsonii	*Drosophila willistoni* group flies	0-3	20
Wolbachia	*Acraea encedon*	61-95	36
	Acraea encedana	95	21
	Adalia bipunctata	0-5	9
Unnamed Flavobacteria	*Coleomegilla maculata*	23	37
	Adonia variegata	13	38
Arsenophonus nasoniae	*Nasonia vitripennis*	4	15
Rickettsia	*Adalia bipunctata*	5-7	6,39
Unknown	*D. bifasciata*	0-7	26
	D. prosaltans	13	33
	Gastrolina depressa	0-81	22
	Epiphyas postvittana	4-7	40
	Hypolimnas bolina	0-61	41
	Spodoptera littoralis	24	42
	Lymantria dispar	9	43

willistoni [20]), and in some exceptional species > 90% of females are infected (e.g., the butterfly *Acraea encedana* [21]). However, there is likely to be study bias towards high-prevalence infections, and all very low-prevalence infections occur in drosophilids, where large samples can easily be bred. Infection prevalence also commonly varies between populations within a host, and prevalence can vary on a remarkably small scale. In the walnut leaf beetle (*Gastrolina depressa*) in Japan, male-killers are absent in populations at the north and south of the islands but present in 50%-80% of females in the center of the islands (22). Prevalence variation on a kilometer scale exists in *Acraea encedon* (21).

Prevalence is determined by the physiologic effect of infection on female host performance, the transmission efficiency of the bacterium from mother to progeny, and the level of advantage to male-killing (determined by host factors such as sibling egg consumption) (Table 2). Transmission efficiency may be influenced by the environment (e.g., high temperatures may lower transmission efficiency), the bacterium, and the host. Selection favors host genes that impede the transmission of the bacteria from mother to progeny. The spread of host resistance genes may prevent infections from commonly reaching the high prevalence achieved by other inherited bacteria.

Table 2. Factors affecting the prevalence of male-killing bacteria

Increase
Decreased rate of inbreeding suffered by female hosts
Increased access to early resources through consumption of dead sibling male hosts
Increased access to resources due to reduced competition, following death of sibling male hosts
Direct physiologic benefits of infection
Decrease
Inefficiency in vertical transmission
Direct physiologic costs of infection
Local extinction of groups having a high prevalence of male-killers

Mechanism of Male-Killing

Little is known about how male-killing is achieved. Neither the cue used to detect sex nor the mechanism by which death is brought about is known in any detail. Indeed, rather than two steps (detection then virulence) there may be only one (constitutive production of a factor that causes death in males only). What we know derives almost exclusively from study of the interaction between *Spiroplasma poulsonii* with *Drosophila*.

Studies of embryos from *D. willistoni* lines infected with *S. poulsonii* show that death occurs at two stages (23): 1) before gastrulation, associated with abnormal cleavage patterns; in particular, achromatic spindles, with other abnormalities of the mitotic process, which account for most embryonic deaths in male-killed lines. 2) After gastrulation, not associated with the normal brown coloration of necrotic embryos; rather, the embryo blackens as a result of breakdown of internal structures and pycnosis of nuclei.

The points of interaction between host and bacterium have been investigated in *D. melanogaster* lines transfected with *S. poulsonii*. In *Drosophila*, sex is determined by the ratio of the X chromosomes to autosomes. In females, which are 2X:2n, the peptide Sxl is produced. Sxl induces female development of the soma and the germ line. In males, which are X: 2n, Sxl is not produced. Absence of Sxl is associated with upregulation of genes on the single X chromosome (dosage compensation), male somatic development, and male germ line development. In *Drosophila*, the male-killer does not interact with any part of the somatic sex development pathway. Mutants of the *tra* gene bear two X chromosomes and produce Sxl but develop as somatic males. They are not, however, killed by *S. poulsonii* (24). Thus, the interaction between male-killer and host is not associated with somatic sex, so the target of detection and virulence is either before Sxl is produced, Sxl itself, or the dosage compensation or germ-line determination pathways.

Although the interaction between *Drosophila* and *S. poulsonii* is the only one studied in any detail, it appears that the mechanism of sex determination exhibited by different male-killer hosts varies widely. Male-killing bacteria have been observed in male heterogametic, female heterogametic, and haplodiploid hosts. Furthermore, members of the same clade of male-killers can be found in hosts of different sex determination systems. The same *Spiroplasma* kills males in ladybirds (male heterogametic) and butterflies (male homogametic). Similarly, male-killing *Wolbachia* have been observed in both male and female heterogametic species

(9). Given that male and female heterogametic systems count chromosomes in opposite directions and show different patterns of dosage compensation, the fact that male-killers operate in both these hosts suggests that the X:autosome counting mechanism and the dosage compensation pathway may not be the focus of male-killing activity; rather, somatic sex determination or germ-line sex determination may be the focus.

Experiments with *S. poulsonii* demonstrate that the somatic sex determination system is not the focus of male-killing behavior. In the case of the other male-killing *Spiroplasma*, the presence of the bacterium in species of different sex determination systems suggests that the focus is either the somatic sex determination or the germ-line determination system. Two conclusions are therefore possible: germ-line determination is the focus of male-killing in all cases, or male-killing has more than one basic mechanism. Further research is clearly warranted.

Direct Effects on Female Hosts

The interaction between male-killing bacteria and their female hosts is interesting. On the one hand, there is selection for a reduction in the number of bacteria present in the host (minimizing virulence) and for a direct physiologic contribution to host metabolism. On the other hand, their fitness is also associated with the fidelity of their transmission to progeny. There may be a trade-off between minimizing virulence and maximizing vertical transmission efficiency, especially if such efficiency is positively related to bacterial number. Thus these bacteria can be either detrimental (if the density of bacteria is high to ensure vertical transmission) or beneficial to the host (if the bacteria play a role in host metabolism).

Empiric studies have suggested that infection usually decreases the performance of female hosts (25,26). The one exception is the interaction between *Spiroplasma poulsonii* and members of the *Drosophila willistoni* group, in which larval development is accelerated by infection (27,28). However, infection is also associated with increased sterility and decreased longevity among adult females (28). Male-killing bacteria, unlike beneficial symbionts, are spread throughout host tissues, and the bacteria may be present in very high numbers. *Drosophila* are infected with extremely high titers of *S. poulsonii* within the hemolymph (29). *Adalia bipunctata* hemocytes are regularly infected with *Rickettsia* (30).

Beneficial effects of male-killing bacteria on host performance cannot yet be ruled out. However, positive effects may be fewer than those found in the "classical" beneficial agents, which typically perform a vital metabolic function that insects are unable to perform. Male-killers infect a minority of females and are rarely carried by larval or adult males. Thus, although they may add to host performance, they cannot substitute for any part of it. A host cannot be dependent on a male-killer for a physiologic function as it can on a beneficial symbiont.

Population and Evolutionary Effects on Hosts

Invasion of a host population by male-killing bacteria affects the dynamics of the host population and alters the pattern of selection on the population to ameliorate the effects of the parasite (Table 3). A high prevalence of male-killers may increase the proportion of female hosts that fail to mate (31), potentially reducing the population size of the host. A dearth of males can subtly alter the mating system of the host. Choice by females of male mates and competition among males for mating opportunities are the

Table 3. Population and evolutionary effects of invasion of a host by male-killing bacteria

Effects on population level	Evolutionary effects
Reduced population density at larval level due to death of male embryos	Selection for increased host clutch size
Failure of females to find mates where parasite prevalence leads to shortage of males, with potential effects on adult population size	Selection for genes that prevent transmission or action of male-killer
Altered epidemiology of sexually transmitted pathogens due to increased reproductive success of males	Alteration in host pattern of sexual selection due to alteration in population sex ratio

rule in insects. However, the biased population sex ratios that result from the spread of male-killing bacteria can reverse this pattern (31). Male choice of females and competition among females for males is expected, with a relaxation of selection on males to ensure paternity.

Male-killers that have invaded populations may cause changes to host biology. Theory predicts selection for an increase in the size of clutch produced (32). Most importantly, genes that prevent the action or transmission of the parasite will be favored. The presence of these genes has been reported (33), but their nature and mode of action are unknown. The means by which insects exclude bacteria is clearly of great import in our understanding of insect-borne diseases, and the nature of resistance genes is expected to be an important focus of future research.

One of the issues to be determined relates to whether male-killing bacteria can cause the extinction of their host. The case of the butterflies *Acraea encedon* and *A. encedana* is suggestive. The *Wolbachia* male-killer in these species is at high prevalence and clearly has some impact on the host population (21,31). If a male-killing bacterium showed perfect vertical transmission, host extinction would be likely. However, selection on the host acts to lower bacterial transmission efficiency, which may ultimately limit the frequency of extinction.

Conclusions: Implications and Uses of Male-Killing Bacteria

Male-killing is an adaptive trait that aids the spread of inherited bacteria through natural populations. The presence of male-killing strains in many bacterial taxa clearly indicates that male-killing should be considered in epidemiologic investigations of vertically transmitted bacteria. Male-killing is perhaps most important in interactions between arthropods and *Rickettsia* and *Spiroplasma*. Members of these genera frequently show horizontal transmission between arthropod hosts (after host-feeding), as well as vertical transmission in the arthropod host. Given that some bacteria in these groups induce male-killing, testing for the presence or absence of this trait should be a part of future investigations of their epidemiology.

The potential usefulness of male-killing bacteria in pest control has yet to be properly assessed. Male-killers may be used on their own

to reduce host population size. Alternatively, they may be integrated into management schemes based on release of sterile males, so that they may amplify the effect of sterile releases on the population size of adult males. In addition, the recent discovery of male-killing in the clade *Wolbachia* adds an extra dimension to the use of this organism in direct and transgenic control of disease transmission.

The usefulness of male-killers in reducing pest damage on their own is debatable. Insect population size and population persistence are largely a function of female, not male, number. Thus, although the presence of a male-killer may reduce larval density, it is unlikely to decrease the population size of breeding females. Furthermore, the presence of density dependence during the larval stages is likely to reduce the effect of male death on numbers of larvae.

Perhaps a more realistic use of male-killing bacteria in pest management would be in conjunction with sterile male release systems of control. In sterile male release, control is achieved through release into the environment of mass-produced sterile males, which mate with females and lower their fertility (34). The success of sterile male release depends on maintaining a high ratio of sterile to normal males in the population. The presence of a male-killer in the host population lowers the number of fertile males and thus increases the effectiveness of any release. The effects of male-killing bacteria at different prevalences on sterile male release, in conjunction with the effects on host population dynamics, need to be investigated. However, direct use of male-killing bacteria as an aid to controlling host numbers is only achievable as a long-term stratagem. Following release of infected hosts into natural populations, spread will occur only in hosts with suitable ecologies and significant prevalence levels will be achieved over a period of years rather than weeks. Another potential application of male-killing bacteria in the sphere of pest and disease vector control may occur indirectly through study of the virulence mechanisms of male-killers.

Perspectives

Dr. Hurst has been a BBSRC David Phillips Fellow at University College London since 1997. His research interests center on the dynamics and importance of parasites that affect insect reproduction.

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