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12 Inherited Microorganisms That Selectively Kill Male Hosts: The Hidden Players of Insect Evolution?

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INTRODUCTION

Contrary to the practice commonly found in genetics laboratories, geneticists in the early and mid-20th century worked frequently with insects taken directly from natural populations. They would cross these, using results to infer the genetic basis of the natural variation they observed. In the course of such experiments, many workers found isofemale lines that produced strongly female-biased sex ratios. Hubert Simmonds, a tropical entomologist, investigating the wing pattern polymorphism of the eggplant butterfly, *Hypolimnas bolina*, incidentally observed lines giving all-female broods (Simmonds, 1923). Ya Ya Lus, investigating elytral pattern polymor-

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phism in *Adalia bipunctata*, the two-spot ladybird, incidentally observed isofemale lines giving all-, or near all-, female broods, in this case associated with lowered egg hatch rate (Lus, 1947). Many workers investigating the genetics of traits in *Drosophila* observed heritable production of female-biased sex ratios, with the affected female again producing eggs with lower hatch rates than found in "normal" females (Magni, 1953; Cavalcanti and Falcao, 1954; Carson, 1956; Malogolowkin, 1958).

Later work established that the cause of each of these female biases was not a nuclear gene but an inherited microorganism living and replicating within the cytoplasm of its host's cells. In each case, the distortion was shown to be associated with the death of male embryos. The causative agents thus became known as son killers or, now more commonly, male killers.

By 1991, a clear pattern had emerged from the cases of male killing on record. There appeared to be two classes of sex-ratio distortion (Hurst, 1991). First, there was distortion associated with the death of male embryos or first-instar larvae. In these cases, the cause was bacterial, which was curable with antibiotics. Second, there was distortion associated with the death of later-instar male larvae. In this case, the agent observed was eukaryotic, members of the phylum Microspora.

In addition to the differences in the agent responsible, work around this time also emphasized differences in the transmission biology of these pathogens. While the embryonic male-killing bacteria were very short-lived in the natural environment and their infectious transmission was very rare, the microsporidia that killed late-instar male larvae were hardy and capable of further transmission following death of their male host. In fact, the death of the male host was associated with the liberation of dispersal spores into the environment. This has led workers to regard the two "strategies" as differing in evolutionary logic, albeit with a common theme (Hurst, 1991) — that these pathogens, which are found in egg cytoplasm but not in sperm, are maternally inherited. Vertical transmission is impossible through a male host, making the death of male hosts at worst neutral for the symbiont. However, the advantage of male-host death differs between the two classes. The principal advantage gained by embryonic male killers is kin-selective, infected female siblings of killed males gain from the death of their brothers. Male-killer-infected neonate female ladybird larvae gain resources by feasting on the soma of their dead male siblings. In contrast, late larval male killing is not adaptive in terms of increasing female host survival. Rather, it allows the pathogen to disperse out of a male host, from which vertical transmission is impossible.

This taxonomic division between differing strategists should not be considered absolute, and it may also be premature to conclude that the list is final. While the hardiness of Microspora in the wild may predispose them to infectious transmission from males (see Chapter 10), there is no obvious obstacle that would prevent microsporidia from being embryonic male killers, and indeed some are known to feminize their hosts (see Chapter 11). Further, there is no reason that cytoplasmic viruses could not cause sex-ratio distortion.

In this review, we first examine the natural history of these two types of sex-ratio distortion, keeping the broad distinction between late and early male killing, which we believe to be intellectually sound, even if likely to be only an approximation of the bacteria/microsporidia lines found to date. We note that the trait of male-specific lethality has evolved independently on many occasions and argue that they are likely to be more common than previously thought, at least in terms of incidence across taxa. With this commonness comes the potential for them to be important in the evolution of a wide range of host taxa. We therefore assess their potential importance in this sphere. How have the biased sex ratios they produce altered the pattern of sexual selection in their hosts? Have hosts evolved to prevent their action, and if so, how? Could they have caused the extinction of host populations or even species? Are they the force that has driven the diversification of insect sex-determination systems? In summary, we propose that these and other sex-ratio-distorting microorganisms may be important hidden players in arthropod evolution.

LATE MALE KILLING

Although the two types of male killing — defined by the timing of male death — appear to be neatly divisible on the basis of the taxonomy of the male-killing agent, bacteria killing early and microsporidia late, the division is best made based on the relative importance of different transmission modes. In early male killing, transmission is almost exclusively vertical. However, late male killing involves a combination of vertical and horizontal transmission, with both being important in the population dynamics of the pathogen on an ecological time scale.

Examples of late male killers are confined to one group of single-celled eukaryotes, the microsporidia. These have been extensively studied in mosquito hosts, which were until recently the only known hosts of late male killers. To date, more than two dozen cases of late male killing by microsporidia have been recorded in mosquitoes. In only a few of these has vertical transmission been demonstrated, with the efficiency of transmission varying from about 0.5 (50% of progeny infected) in *Aedes stimulans* (Andreadis, 1985) to more than 0.9 in *Culex salinarius* (Andreadis and Hall, 1979). Reported prevalence levels typically range from 0.02 to 0.4. Early workers noted that they could not be maintained purely by transovarial transmission at the observed rates and therefore suggested that horizontal transmission must be occurring (Andreadis and Hall, 1979; Lord, et al., 1981). This was subsequently demonstrated in a number of cases (Andreadis, 1985; Avery, 1989; Sweeney et al., 1985, 1988; Becnel, 1986).

In the simplest scenario, the microsporidia in female mosquitoes are transovarially transmitted to offspring. Those in males, for which vertical transmission is not an option, may be horizontally transmitted to other hosts either via larval cannibalism or when spores are released into the water following the death of their host, which typically occurs when the larva is in its final instar. Released spores may be ingested by other mosquito larvae either directly or within a copepod intermediary, which may become infected by the spores and subsequently preyed upon by the mosquito larvae (Becnel and Sweeney, 1990). Both male and female larvae can be infected horizontally. Neither shows ill effects following novel infection within their own lives. Indeed, no phenotypic effects of the microsporidia have been reported for newly infected males. These males complete their life cycle normally, and the microsporidia that they now contain die when their host dies. Newly infected female hosts also complete their life cycle normally, but these pass the microsporidian into their eggs. The males in this following generation may be killed while females continue to transmit the symbionts vertically.

In nature, the situation is often more complex than this simplest scenario. Some microsporidians cause the death of both host sexes, while others kill neither (see Chapter 10). Kellen et al. (1965) detailed four types of infection of mosquitoes by microsporidia, the types being differentiated largely on the level of pathogenic effects of the microsporidia in the host sexes (Table 12.1).

Kellen et al. (1965) argue that Type III infections are a primitive state. In laboratory experiments, Sweeney et al. (1989) showed that the survival rate of female *C. incidens* and *C. inornata* to adulthood was less than 2% and that this figure varied between species. Moreover, the survival rate responded to artificial selection, implying a genetic component to the *modus operandi* of the microsporidian: either killing female hosts or being transovarially transmitted. One may then speculate that Type III infections will evolve into Type II, then Type I, and, perhaps, finally Type IV infections, as vertical transmission becomes predominant over horizontal transmission. The relative efficiencies of horizontal and vertical transmission will depend not only on the pathological characteristics of the microsporidian but also on host density and the availability of intermediate hosts.

The variations between the types detailed by Kellen et al. (1965) beg several questions. How do the microsporidians kill their hosts? How do some selectively kill male rather than female hosts? Why do host deaths usually occur precisely in the fourth larval instar? Why do different microsporidians employ different strategies, some being pathogenic to both sexes, some just to males, and some to neither sex? Not all of these questions have clear answers. In particular, the methods by

TABLE 12.1
Classification of Sex-Related Pathogenicity of Microsporidia in Mosquitoes

	Effect on Male Hosts	Effect on Female Hosts
Type I	Death in fourth instar due to progressive infection following sporogeny	Transovarial transmission; no reduction in fecundity of egg hatch rate
Type II	Death in fourth instar due to progressive infection following sporogeny	Some but not all infected females die in the fourth instar due to progressive infection following sporogeny; not reported whether surviving females transmit transovarially
Type III	Death in fourth instar due to progressive infection following sporogeny	Most die in fourth instar due to progressive infection following sporogeny; however, a few survive sporogeny to adulthood and transmit symbiont transovarially
Type IV	Infection limited to small regions of thoracic and abdominal adipose tissue; not lethal	Infection limited to small regions of thoracic and abdominal adipose tissue; not lethal; transovarially transmitted

Source: Data from Kellen, W.R. et al. (1965). *J. Invertebr. Pathol.* **7:** 161.

which microsporidians determine the sex of their host and by which they cause their host's death await discovery.

Hurst (1991) argued that the timing of host death was crucial if the death of the host was coupled with horizontal transmission. By causing death in the fourth larval instar the microsporidian maximizes its transmission rate. He suggests, justifiably, that horizontal transmission needs to occur in water. Further, he argues that the pupal case, the last aquatic stage of the life cycle, might act as a barrier to symbiont release. In consequence, he states that causing host death in the final larval instar, but not before, maximizes the number of microsporidian spores released into the water to be taken up by copepods that would then vector the microsporidian if preyed upon by other mosquito larvae.

Before last year, all known cases of late male killing involved microsporidia parasites in mosquito hosts. The recent observation of late male killing in the oriental tea tortrix moth, *Homona magnanima*, suggests it is premature to confine the phenotype of late male killing to aquatic insects. In the oriental tea tortrix, larval mortality was observed in all instars, with greatest mortality during the third instar (Morimoto et al., 2001). Total larval mortality in lines producing mainly females was around 50%, compared to 10% in normal lines. Feeding homogenate of dead larvae from female-biased lines to uninfected larvae successfully effected horizontal transmission. The trait was resistant to antibiotic treatment. The combination of the timing of male death with the ability to transfer horizontally makes a compelling case for the idea that late male killing occurs in the terrestrial environment, with the male killing similar in logic to that in mosquitoes.

A general consideration of insect pathology further suggests it is premature to confine late male killing to microsporidia as etiologic agents (Majerus, 2002; Stouthamer et al., 2002). Vertical transmission of pathogenic microorganisms in insects was first recorded by Louis Pasteur (1870), who found that the microsporidian responsible for pebrine disease in the silkworm, *Bombyx mori*, was transmitted both horizontally from dead or dying larvae and vertically in the cytoplasm of the eggs. Since then, vertical and horizontal transmission has been recorded in other parasites, such as nuclear polyhedrosis viruses, which are known to persist for over 25 years within polyhedra crystals exposed on plant material. If then ingested by appropriate lepidopteran larvae, the crystals are broken down in the gut and the virus migrates to cells where it replicates. As we will show, cannibalism and intraspecific consumption play an important part in the dynamics of early male killing. It is possible that intraspecific consumption or cannibalism also has a role to play in late

male killing if horizontal transmission is facilitated. In some genera of moths (*Spodoptera, Mamestra, Melanchra*), larvae are attracted to fresh corpses of viral-killed conspecifics when the integument ruptures and then feed on the liquified remains, thereby imbibing high doses of the viral pathogen.

EARLY MALE KILLING

Early male killers present several contrasts to late male killers. First, all early male killers described to date are bacterial. They have been recorded from a phylogenetically wide range of hosts. Most significantly, early male killers are maternally transmitted, and horizontal or paternal transmission is usually rare or absent. This is known both directly from experiments that have looked for horizontal transmission (see Hurst and Majerus, 1993 for review) and indirectly as male-killing bacteria have been observed to be in linkage disequilibrium with maternally inherited host genes in the mitochondria (von der Schulenburg et al., 2002). The lack of horizontal transmission is probably at least in part linked to the fact that the causal agents are bacterial. As has been noted elsewhere in this book, inherited bacteria are generally very refractory to culture and show poor survival outside of host cells. While horizontal transmission on blood–blood contact is feasible (though likely to be limited in rate by the frequency of contact), transmission through the environment is unlikely.

The lack of horizontal transmission of early male killers out of the dead male embryo suggests both that there is some other function to male lethality and that some other force is maintaining the agents in the population. It is thought that these male killers are maintained because the death of males increases the survival and reproductive success of their sisters. These females bear the same bacterium by descent and will transmit it vertically. The increase in female fitness resulting from the death of males has been termed "fitness compensation" and can occur for three reasons: a reduction in the rate of inbreeding and consequent fitness losses through inbreeding depression, resource reallocation, and a reduction in the cannibalism of females themselves (Skinner, 1985; Werren, 1987; Hurst, 1991; Hurst et al., 1992). These are not mutually exclusive.

If a host species is prone to inbreeding, then male killing can decrease the rate at which infected females inbreed (they simply have no brothers to mate with) (Werren, 1987). If inbreeding is deleterious, then this will increase the reproductive success of infected females, too. The avoidance of inbreeding therefore represents a benefit to male killing that may occur in many different types of host, although it is perhaps unlikely to be a common benefit, as high rates of inbreeding are generally uncommon outside the Hymenoptera and other haplodiploid taxa. Within the Hymenoptera, inbreeding is more common, although the haplodiploid genetic system within this group means inbreeding depression is rarely severe.

The spread of a male killer as a result of resource reallocation relies on the resources made available to females from the death of males being preferentially available to infected females. The greatest benefit will accrue to surviving progeny of infected females when offspring from one mother occur close together. Thus, species that produce large clutches of eggs together will be more prone to the spread of early male killers than those that disperse their offspring. The benefits associated with resource reallocation can be fairly large and vary among arthropod taxa depending on their ecology. From this feature alone, the expectation is that the distribution of male-killer hosts will not be random taxonomically.

Host Diversity

Early-male-killing bacteria have been recorded from a taxonomically diverse array of hosts from five orders of insects and from two species of mite. Therefore, mechanistic constraints do not appear to confine them to a narrow range of arthropod taxa. Instead, their distribution is determined primarily by the ecological factors discussed above (Hurst and Majerus, 1993). Hot spots for male

killing are known in the milkweed bugs (Hemiptera: Lygaeidae), nymphalid butterflies, particularly of the genus *Acraea* (Jiggins et al., 2001a), and the ladybird beetles (Coleoptera: Coccinellidae) (Majerus and Hurst, 1997). These hot spots are associated with aspects of host ecology that make male killing beneficial. The best-studied group in this regard is the ladybirds.

Three aspects of the biology of aphidophagous ladybirds underlie the high incidence of male-killer infection in this group. First, aphidophagous ladybirds lay eggs in tight clutches. Second, aphids are prone to rapid population increases and crashes so that they are a highly ephemeral prey for ladybirds. Third, ladybirds are highly cannibalistic; in particular, they indulge in sibling-egg cannibalism/consumption. These facts are not independent; prey ephemerality promotes sibling-egg cannibalism, which in turn causes rapid embryonic development (Majerus and Majerus, 1997). The result is that neonate ladybird larvae are very small and have minimal energy reserves when they hatch. Starvation rates of neonate aphidophagous coccinellid larvae are often very high (Banks, 1955, 1956; Wratten, 1973). Comparison of neonate larvae from clutches laid by male-killer-infected and uninfected *A. bipunctata* females showed that the former survived half as long again as the latter after dispersal from their natal egg clutch when denied food or water. Furthermore, larvae from male-killed clutches were larger at dispersal and could subdue a greater size range of aphids and travel further in search of food before dying from starvation than larvae from normal clutches (Hurst, 1993). The greater resources consumed by such larvae before they dispersed from their egg clutches led to more rapid development and higher likelihood of survival to first ecdysis.

Similar or greater advantages resulting from resource reallocation via sibling-egg consumption have been shown in other coccinellids. Here, then, by their sacrificial suicide, the bacteria in male eggs increase the fitness of clonally identical copies of themselves in female siblings of their hosts: an exquisite and extreme case of kin selection.

A second advantage to male killing dependent on the cannibalistic behavior of neonate ladybird beetle larvae entails the reduction in the probability that slow-developing female larvae in male-killed clutches will be cannibalized by faster-developing siblings. This reduced probability is a consequence of both the smaller number of larvae that hatch and the greater number of unhatched eggs available to early-hatching larvae in a male-killed clutch than in a normal one. Evidence that this reduction in cannibalism leads to an increase in the number of female larvae that hatch in male-killed compared to normal clutches has been obtained for two species of coccinellid, *A. bipunctata* and *Coccinula sinensis* (Hurst, 1993; Majerus, 2001).

A third potential advantage to male killing arising from larval cannibalism has yet to be verified by empirical study. Not only do larvae consume unhatched eggs in their clutch before dispersal, but they will also eat conspecific larvae once they have dispersed, particularly if other manageable prey is scarce. In interactions between two larvae, assuming that neither is restricted by ecdysis, the larger larva usually wins and eats the smaller (Majerus, 1994). As larvae from male-killed clutches are on average larger than normal larvae when they disperse, the larvae from male-killed clutches are likely to gain another cannibalistic advantage when clutches of eggs are laid close together and at the same time by infected and uninfected females.

The cannibalistic behavior of aphidophagous ladybirds, coupled with their habit of laying eggs in batches and the ephemerality of their prey, underlies their susceptibility to male-killing symbionts. In this case, the advantage from male killing results primarily from the redistribution of resources from the killed males to their sisters that have a high likelihood of carrying the same male-killer lineage of bacteria.

The evolutionary rationale underlying the widespread occurrence of male killers in aphidophagous coccinellids is well understood. In other species in which early male killers have been recorded, reasons are less clear, although circumstantial evidence based on the ecologies of the host insects suggests a resource advantage to male killing in some cases (Hurst and Majerus, 1993). However, some cases still represent something of a conundrum. The male-killing bacteria found in *Drosophila* are one case; a resource advantage associated with male killing is not obvious. The butterfly *Danaus chrysippus* represents another ambiguous case (Jiggins et al., 2000a). Here, eggs

are laid singly, and antagonistic interactions between sibling larvae appear unlikely; thus, a significant resource advantage from the death of infected males to infected female siblings appears impossible. Clearly, further studies of potential sources of fitness compensation are needed in these butterflies. In general, it is proposed that these sporadic cases are maintained from advantages associated with reduced rates of inbreeding, but little or no data are available to corroborate this.

Potential Other Hosts of Early Male Killers

Early-male-killing bacteria have been reported from a diverse range of insect hosts, with some families of insect appearing to be particularly prone to male-killer invasion as a consequence of their ecology and behavior. It is likely that the list of hosts is, as yet, extremely incomplete. Certainly many other instances of male-killer infection will be detected in the known hot spots. For example, it is possible to make a rough estimate of the number of Coccinellidae likely to harbor male killers. Taking into account the approximate number of species in the family (6000), the proportion that are aphidophagous, lay eggs in clutches, and indulge in sibling-egg consumption/cannibalism (0.2) and the proportion of such species assayed for male killers that have been found to bear them (0.59) produce a figure of more than 500 host species. Furthermore, this figure does not take into account incomplete ascertainment, which in species where the number of matrilines assayed were low may be significant due to the low prevalence of many male killers.

The characteristics thought to promote male-killer invasion in known "hot-spot" groups can be used to predict other groups likely to bear male killers. The necessity in models of male killing for benefits accruing from the death of males to be preferentially conferred upon infected compared to uninfected females means that fitness compensation sufficient to allow the persistence of a male killer is most likely in species in which antagonistic interactions among siblings are common. These antagonistic interactions may be direct through cannibalism or indirect through competition for nutrients or other resources. A number of groups of insects and other arthropods, from which male killers have not previously been reported, would appear to be likely candidates for male-killer infection. These include aphidophagous lacewings (Neuroptera), web spinners of the order Embioptera, some gall-forming Diptera and Hymenoptera, some predatory Hemiptera, some mantids, and many spiders. On average, sibling interactions are more common in aposematic species than in other species simply because of the high proportion of aposematic species that remain in family groups during their immature stages. Consequently, male killers are more likely to occur in groups with a high proportion of aposematic species.

The Systematic Diversity of Male-Killing Bacteria

In all cases where the causative agents of early male killing have been identified, they have proved to be bacteria. Initially these studies consisted mostly of curing the trait using antibiotics or heat, and further identification was hampered because the bacteria could not be cultured. However, the advent of polymerase-chain-reaction (PCR)-based technology, particularly the sequencing of 16S ribosomal DNA, has revealed male-killing bacteria to be remarkably diverse.

The male-killing bacteria belong to various distantly related bacterial taxa (Table 12.2). From these data we can conclude that male killing must have evolved at least six times — twice in the α-proteobacteria, once in the γ-proteobacteria, twice in the *Spiroplasma*, and once in the Flavobacteria. These independent origins of male killing have occurred in bacterial groups with a variety of different ecologies. The members of all these bacterial groups are associated with arthropods, but they differ both in whether sister associations are mutualistic or parasitic and in whether they are vertically or horizontally transmitted (Hurst and Jiggins, 2000). The majority of species of *Rickettsia* and *Spiroplasma* are arthropod-vectored diseases of either plants (*Spiroplasma*) or vertebrates (*Rickettsia*) (Whitcomb, 1980; Winkler, 1990). In contrast, other arthropod symbionts in the genus *Wolbachia* are mostly vertically transmitted parasites that manipulate the reproduction of their hosts, often by distorting the primary sex ratio (Stouthamer et al., 1999).

TABLE 12.2
The Systematic Diversity of Early Male-Killing Bacteria and Their Hosts

Bacterium		Host	Ref.
γ-Proteobacteria	*Arsenophonus nasoniae* (Enterobacteriaceae)	*Nasonia vitripennis* (Hymenoptera)	Werren et al., 1986
α-Proteobacteria	*Rickettsia typhi* relative (Rickettsiaceae)	*Adalia bipunctata* (Coleoptera)	Werren et al., 1994
		Adalia decempunctata (Coleoptera)	von der Schulenburg et al., 2001
		Brachys tesselatus (Coleoptera)	Lawson et al., 2001
	Wolbachia	*Adalia bipunctata* (Coleoptera)	Hurst et al., 1999c
		Tribolium madens (Coleoptera)	Fialho and Stevens, 2000
		Acraea encedon (Lepidoptera)	Hurst et al., 1999c
		Acraea encedana (Lepidoptera)	Jiggins et al., 2000b
		Drosophila bifasciata (Diptera)	Hurst et al., 2000
		Hypolimnas bolina (Lepidoptera)	Dyson et al., 2002
Flavobacteria	*Blattabacterium* relative	*Coleomegilla maculata* (Coleoptera)	Hurst et al., 1997b
		Adonia variegata (Coleoptera)	Hurst et al., 1999b
Mollicutes	*Spiroplasma ixodetis* relative	*Adalia bipunctata* (Coleoptera)	Hurst et al., 1999a
		Harmonia axyridis (Coleoptera)	Majerus et al., 1999
		Danaus chrysippus (Lepidoptera)	Jiggins et al., 2000a
	Spiroplasma poulsonii	*Drosophila willistoni* (Diptera)	Williamson et al., 1999

Finally, *Blattabacterium* is a vertically transmitted mutualist found in cockroaches and termites (Bandi et al., 1994), and *Arsenophonus nasoniae* is related to inherited bacteria isolated from the assassin bug *Triatoma* (Hypsa and Dale, 1997) and bacteria of unknown transmission biology from the psyllid *Diaphorina citri* (Subandiyah et al., 2000) and whitefly *Aleurodicus dugesi* (Spaulding and von Dohlen, 2001).

The male-killing taxa also differ in their location within the host. For example, *Spiroplasma poulsonii* is largely extracellular, while *Wolbachia* and *Rickettsia* are both found predominantly within the host-cell cytoplasm. Unlike mutualistic bacteria, which are often found in specialized tissues or organs, there is no evidence that male killers are restricted to specific tissues, and they are typically present in the hemocytes (Hurst et al., 1996a).

To what extent does the sample in Table 12.2 reflect the total diversity of male-killing bacteria? An indication of their total diversity can be gained by reviewing studies that first established the presence of male killers from their phenotypic effects and then identified the bacterium (as opposed to vice versa). This is shown graphically in Figure 12.1, which plots the rate at which new bacterial taxa of male killers have been discovered against the rate at which new male killers have been

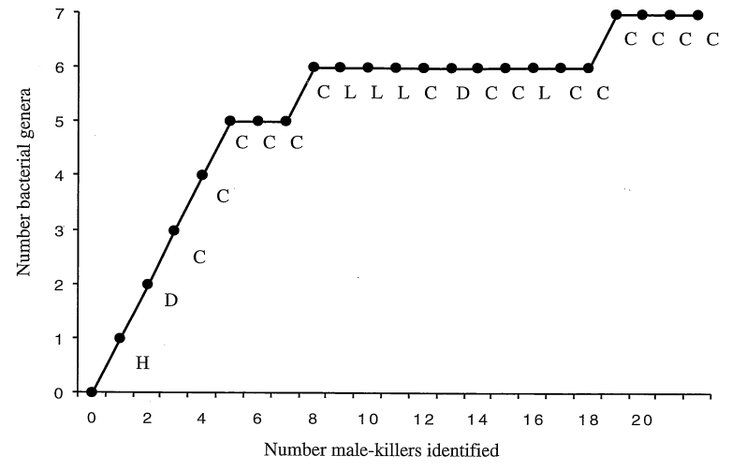

FIGURE 12.1 The rate at which new genera of male-killing bacteria have been described plotted against the number of male killer–host interactions where the bacterium has been identified. Two *Spiroplasma* clades are included as separate genera as this genus is paraphyletic. The date of the description of a new male killer was taken as the date when DNA sequences were published, which confirmed its taxonomic identity. The identity of the host is included as C = Coleoptera, D = Diptera, H = Hymenoptera, and L = Lepidoptera.

identified. Two conclusions can be drawn from this graph and Table 12.2. First, the majority of recent identifications belong to bacterial taxa already known to contain male killers, and therefore the total diversity of male killers in insects is unlikely to be orders of magnitude greater than that already described. Second, when new orders of insects are investigated, they are often found to be infected with bacteria closely related to those found in different orders. Therefore, there is surprisingly little evidence of any systematic variation in the causative agents of male killing across host taxa. If our unpublished records are included, then representatives of all the major bacterial groups in Table 12.2 have been recorded killing males in the best-studied insect family, the Coccinellidae.

Another pattern evident in Table 12.2 is that closely related bacteria are commonly found in distantly related hosts. Therefore, these bacteria must have switched hosts during their evolution. For example, the spiroplasmas that cause male killing in beetles (*A. bipunctata*, *Harmonia axyridis*) and butterflies (*D. chrysippus*) are phylogenetically more similar than their hosts (von der Schulenburg et al., 2000), suggesting that horizontal transmission between species has occurred. Artificial horizontal transmission of the *H. axyridis* male killer into the related beetle *A. bipunctata* produces a male-killing phenotype. Therefore, while horizontal transmission of early male killers is probably of little importance over ecological time, it may be crucial over evolutionary time. Does this mean that all male killers can survive and kill males across many different insect taxa? The only experimental study investigating the ability to transmit over wide phylogenetic distances of host range suggests that this is not the case. When *Spiroplasma poulsonii* and related bacteria that are male killers in the *D. willistoni* species group were injected into other species in the genus *Drosophila,* the majority (although not all) of the recipient species stably transmitted the infection and expressed the trait (Williamson and Poulson, 1979). However, in the more distantly related housefly, *Musca domestica,* the bacterium persisted in the hemolymph but was not transmitted transovarially (Williamson and Poulson, 1979). When the bacterium was injected into species from different insect orders, it was rapidly eliminated from the hemolymph (Williamson and Poulson, 1979). Thus, although transmission between disparate hosts can occur, as witnessed by the *Spiroplasma* in beetles and butterflies (Hurst et al., 1999a; Jiggins et al., 2000a), increasing phylogenetic distance between hosts does represent an increasing barrier to horizontal transmission.

Another intriguing question posed by the data in Table 12.2 is whether the mechanisms of male killing are the same in different host and bacterial taxa. This reflects perhaps the largest gap in our understanding of male killers, but two points are worthy of mention. First, related male-killing bacteria can be found in hosts with very different sex-determination mechanisms. Both beetles and butterflies contain related strains of *Spiroplasma* and *Wolbachia* that kill male hosts. These hosts have very different chromosomal sex-determination systems, beetles being male heterogametic and butterflies female heterogametic. This suggests that either these bacteria can have adapted to new sex-determination systems or that they use some common element of sex determination. This would be intriguing, given the wide diversification of insect sex-determination systems observed: to date there are no "common" mechanisms known. Second, it is unknown whether male death occurs because bacteria detect host sex and then express a second "toxin" gene or whether the bacteria systemically produce a toxin that acts only in males, interfering, for instance, with male sex determination. In this latter context, it is notable that many mutations in the sex-determination pathway are not embryonic lethal. In *Drosophila*, for instance, mutations altering *Sxl* expression are embryonic lethal, but those involved with somatic sex and dosage compensation are not. Thus, direct interference by bacteria will not produce male death. If the bacterium is interfering with sex determination itself, it must be doing so in a fairly fundamental fashion, at the level of the key switch genes. Otherwise, male killing is a response to differences in the expression of genes within the sex-determination pathway without itself being interference of it.

Variation in the Prevalence of Early Male Killers in Natural Populations

Male-killing bacteria are present in a wide variety of species, but their prevalence varies among species. Prevalence is commonly low in drosophilids (1 to 20% of females infected), varies from low (7%) to high (50%+) in ladybirds (Hurst et al., 1993; Majerus et al., 1998), and varies from low (4%) to extremely high (>90% females infected) in Lepidoptera (Geier et al., 1978; Jiggins et al., 1998). Intraspecific variation can also be profound. In the chrysomelid *Gastrolina depressa*, the ladybird *H. axyridis*, and the butterfly *D. chrysippus*, some populations are uninfected, but 40 to 60% of females are infected in others (Chang et al., 1991; Majerus et al., 1998; Jiggins et al., 2000a). In *A. encedon*, prevalence varies from 65 to 95% over relatively short ranges.

What is the cause of this variation in prevalence? The prevalence of male-killing bacteria in natural populations depends on the vertical-transmission efficiency of the male killer, the direct fitness effects of male killers on female hosts, and the extent of fitness compensation. In a minimal model in the absence of inbreeding effects (Hurst et al., 1997a), invasion is possible if $b > 1/(a(1 + s)) > 1$, where b is the benefit accruing to infected females as a result of the death of males (a benefit exists where $b > 1$), a is the vertical transmission of the male killer ($0 < a \leq 1$), and s is the direct effect of bearing a male killer on female fitness ($s > 0$ is a beneficial effect and $s < 0$ is a deleterious effect of infection). Following invasion, equilibrium prevalence is given by $p^* = (ab(1 + s) - 1)/(b(1 + as) - 1)$.

Therefore, these three factors are the most likely causes of the variation in prevalence we observe within and among species. We can investigate each of these in turn.

Transmission Fidelity

As is the case for late male killers, early male killers are not transmitted to all the offspring of infected females. Although some male killers have high vertical-transmission efficiencies (>0.99), laboratory assessments of the vertical-transmission efficiencies of most male killers give lower values, typically in the range 0.8 to 0.95 (Hurst and Majerus, 1993). Their rate of transmission contrasts with the higher figures found for *Wolbachia* that induce cytoplasmic incompatibility and the lower figures found for late male killers.

Variation in the rate of vertical transmission is observed in different male-killing bacteria found within a single host species. For instance, in *A. bipunctata* different male killers have different

TABLE 12.3
Vertical Transmission Efficiencies of Male-Killing Bacteria in Six Matrilines from Moscow

Line	Number of Families	Total Progeny	Sex Ratio (proportion males)	Mean Vertical Transmission Efficiency
Mos 3 (*Rickettsia*)	5	123	0.220	0.719
Mos 6 (*Wolbachia* Z)	6	222	0.126	0.856
Mos 9 (*Spiroplasma*)	6	130	0.015	0.984
Mos 18 (*Wolbachia* Y)	7	168	0.012	0.988
Mos 33 (*Spiroplasma*)	4	170	0.006	0.994
Mos 35 (*Spiroplasma*)	5	199	0	1

Source: Data from Majerus, M.E.N., von der Schulenburg, J.H.G., and Zakharov, I.A. (2000). *Heredity* **84:** 605.

vertical-transmission efficiencies within the same host (Table 12.3). This suggests that the vertical-transmission efficiency of these bacteria is a function of the male killer itself and not of the host. In contrast, the *Wolbachia* strain that infects both *A. encedana* and *A. encedon* in Uganda, which appear to be identical (no differences observed in more than 5000 nucleotides of bacterial and mitochondrial DNA sequenced), have a vertical-transmission efficiency of 0.96 in the former and 1.0 in the latter, suggesting that vertical-transmission efficiency may in part be a function of the host. In reality, the variation in transmission efficiencies is likely to be a product of interaction between the symbiont, its host, and the environment.

Inefficient transmission is thought to be the main factor that results in stable polymorphisms of infected and uninfected females in a population (Hurst, 1991). If a male killer can invade a population and is transmitted with perfect fidelity to the next generation, it will spread to fixation, potentially driving the host extinct (see below). However, if transmission is imperfect, then uninfected females and males are produced by infected mothers, which can result in a stable polymorphism of infected and uninfected females. The causes of this imperfect transmission are unknown, but possible explanations include environmental curing (Hurst et al., 2000), some form of host immune response, or a possible tradeoff between transmission efficiency and some other aspect of bacterial fitness (see below).

Direct Effects on Female Host Survival and Fecundity

Male killers rely on the survival and reproduction of female hosts to be transmitted and are therefore selected to have low virulence in female hosts. While assessments of the direct fitness effects of harboring male killers are sparse, in the majority of those that have been undertaken, costs rather than benefits have been found. In three species of ladybird beetle carrying different male killers (*A. bipunctata* — *Rickettsia*; *H. axyridis* — *Spiroplasma*; *Adonia variegata* — Flavobacterium), costs in terms of decreased rate of egg laying, higher levels of infertility, and shorter adult life span have been observed (Matsuka et al., 1975; Hurst et al., 1994, 1999). In *D. willistoni*, fertility and female embryo survival are reduced (Ebbert, 1991), and in *S. littoralis* and *E. postvittana*, fecundity is reduced (Geier et al., 1978; Brimacombe, 1980).

Recent work on *Wolbachia* in nematodes and insects has suggested the presence of positive physiological advantages to possessing inherited bacteria, even ones that are reproductive parasites. This is certainly a possibility for male-killing bacteria, although definitive data are yet to be obtained. More rapid larval development has been reported in *D. willistoni* and *D. nebulosa* infected with *S. poulsoni* (Malogolowkin-Cohen and Rodriguez-Pereira, 1975; Ebbert, 1991), though it should be noted that infection is associated with an increased frequency of host sterility in this interaction.

Why is it that experiments have generally found a cost to infection, when a "Darwinian Demon" male killer would have either no cost or a benefit to infection? One possible evolutionary scenario is that the virulence of the male killer is correlated to some other trait affecting parasite fitness, such as transmission efficiency (Hurst et al., 1994). If transmission efficiency and bacterial titer are correlated, it is easy to see how natural selection could produce a costly bacterium because this was more efficiently transmitted. It may also explain why transmission efficiency is rarely perfect.

Level of Fitness Compensation

As discussed earlier, fitness compensation is the increase in lifetime fecundity of females that results from the death of males. This is the principal factor responsible for driving up male-killer frequency. While it is easy to get a broad view of differences in the magnitude of this factor (it is fairly noncontentious that the incidence and prevalence of male killing are much higher in aphidophagous coccinellids than in *Drosophila* because the former show egg cannibalism), it is hard to gain any absolute measure (Hurst and Majerus, 1993). This is because the magnitude of this factor is ecologically dependent. How much eating an egg increases ladybird survival depends on the local aphid density, which varies greatly within and between years and over space. Measurement must be performed in the field and varies from being very hard to measure to nearly impossible.

An idea of the magnitude of these effects can be gained from scoring prevalence of the agent and then measuring transmission efficiency and costs of infection. What is left is the likely magnitude of any direct benefit. For instance, 7% of *A. bipunctata* in Cambridge, U.K. are infected with male-killing *Rickettsia*, which has a transmission fidelity of around 87%. To achieve this prevalence with this transmission efficiency requires that an infected female produce around 1.16 times as many surviving daughters as an uninfected female (Hurst et al., 1993). Given that there is around a 10% cost of infection in the absence of any benefit from the death of males (Hurst et al., 1994), this leaves us estimating an increase in female fitness resulting from male death of around 28%.

This process was also conducted for the male-killing *Wolbachia* in *A. encedana*, where prevalence is very high (96% of females infected). Jiggins et al. (2000b) obtained an estimate of the vertical-transmission efficiency of the *Wolbachia* male killer in *A. encedana* by collecting larval nests from the wild and testing individual larvae for *Wolbachia* presence or absence. The vertical-transmission efficiency was estimated to be 0.96. On the basis of this estimate, and assuming that the male killer was at equilibrium, the lifetime reproductive output of infected relative to uninfected females was estimated to be at least half as great again (minimum estimate within 95% confidence intervals = 1.55). This is an extraordinarily high estimate, and it left the authors to conclude that there might be a direct positive effect of infection on female fitness.

Perhaps more tractable is the measurement of the effect of male killing on losses from inbreeding depression. In only two cases have data been sought specifically on this point. In *A. bipunctata*, the level of inbreeding in the wild was measured from egg clutches and found to be rare (Hurst et al., 1996b). In the gypsy moth, *Lymantria dispar*, which is host to a male killer with high vertical-transmission efficiency (Higashiru et al., 1999), a similar approach again showed inbreeding levels to be low. With the establishment of neutral genetic markers in a wide variety of species, measurement of inbreeding rates in the field should become more routine. It should be possible to test whether the spatial variation in male-killer prevalence in *D. chrysippus* or *D. bifasciata* is associated with variation in inbreeding rates.

In conclusion, it is clear there is variation in the magnitude of the effect that male death has on sibling-female survival, and this will be responsible for some of the variation in prevalence observed within species. However, variation in prevalence of different male killers within a locality, as is observed in *A. bipunctata* from Moscow (see below), indicates that other factors above (transmission efficiency, cost) are also important in producing the variation in prevalence we observed among and within species.

Coexistence of Male Killers within a Population

Minimal models of male-killer dynamics, based on vertical-transmission efficiency, direct-fitness effects on female hosts, and fitness compensation, predict that long-term coexistence of multiple male killers in a host population is not possible. Yet this prediction is undermined by the finding of four different male killers (a *Rickettsia*, a *Spiroplasma*, and two *Wolbachia*) in a Muscovite population of *A. bipunctata* (Majerus et al., 2000) and the observation of distinct *Wolbachia* strains in *A. encedon* (Jiggins et al., 2001b).

It is possible that these are stable polymorphisms maintained by natural selection. For example, Randerson et al. (2000a) have shown that two male killers can coexist if the host evolves partial resistance to male killers with the higher product of transmission efficiency and cost. Although no evidence of male-killer suppression has been obtained in *A. bipunctata*, and the parameter space representing coexistence is limited in the Randerson et al. model, their result does suggest that at least two male killers may coexist at equilibrium in a host population. Multiple male killers could also be maintained due to spatial heterogeneity, with different male killers favored in different patches coupled with migration between these patches. Alternatively, we may be observing a dynamic system that rarely, if ever, reaches equilibrium. Simulations suggest that the replacement of one male killer by another more competitive male killer can sometimes take thousands of generations. The four male killers in Muscovite *A. bipunctata* may be the result of migration, temporal variance in the selective pressures on each male-killer strain, and slow reduction in prevalence of the less-fit strains.

Consequences of Male-Killing Bacteria for the Dynamics and Evolution of Their Hosts

Male-killing bacteria can be common within a species. As they become more common, the sex ratio in the population becomes more female biased, and this may have effects on female fertility and alter patterns of sexual selection. Additionally, they can produce very strong selection for genes in the host population that prevent their action or transmission or otherwise ameliorate the effects of their presence. Here they may become very important evolutionary agents because of the sheer magnitude of selective pressures they can generate. We argue that prevalence can reach such extreme levels that they can select for genes that are usually very costly. As such, they have the potential to be a real source of evolutionary novelty, for instance, in the evolution of sex-determination systems.

Could Male-Killing Bacteria Lower Population Size or Cause Population Extinction?

In 1967, Hamilton (1967) noted that selfish genetic elements that distort the sex ratio could result in the extinction of entire populations or species through lack of the sex against which the selfish genetic element drove. He considered the case of a meiotically driving X chromosome and noted that as the driving element reached higher and higher frequency, the sex ratio became increasingly female-biased, and there would come a point at which there were insufficient males to fertilize all the females within the population. Ultimately, the population would progressively shrink in size, making possible the extinction of the population bearing the element.

Hatcher et al. (1999) have examined in greater detail the effects of inherited parasites that feminize their hosts. They noted that the point at which population decline occurred depended on two factors. The first factor is the extent to which a male can mate multiply. If a single male is able to fertilize many females, then female unmatedness will occur only at extreme population-sex-ratio biases. The second factor is the strength of density-dependent factors that regulate population size. The effects of unmatedness of females due to lack of males would be buffered at the

population level by decreased density-dependent mortality. In simple terms, larvae from different females usually compete, and this interference causes a certain degree of larval mortality or reduced fecundity of adults. The unmatedness of some females in extremely sex-biased populations is therefore directly compensated at the population level by increased survival and fecundity of the progeny of others.

The above papers explicitly modeled cases of meiotic drive and feminization, but the principle (that sex-ratio-distorting elements transmitted with high fidelity and with strong sex-ratio distortion can damage populations) holds true across classes of selfish genetic element. Indeed, Hamilton used the case of the female-biased sex ratios in *A. encedon* as evidence for his notion that driving sex chromosomes could cause population-level effects. This system is now known to be one where strongly female-biased population sex ratios are associated with high prevalence of male-killing *Wolbachia*, not sex-chromosome drive. But how commonly might male killers place a population at risk of extinction?

It is hard to evaluate how commonly male killers cause population extinction, as this is an outcome that we would be unlikely to observe. Indeed, Stouthamer et al. (2002) argue that this unobservable process may be a likely outcome. They take as evidence for this the observation that parthenogenesis-inducing microorganisms can become fixed in natural populations. They note that the parallel dynamic acting in the case of a sex-ratio distorter, rather than a distorter of sexuality, would be population extinction, although they caution that what is true of parthenogenesis induction does not necessarily hold true of all classes of sex-ratio distorter. However, there are two reasons to believe this process is unlikely to occur for a male-killing microorganism. First, the drive associated with male death (up to a 55% increase in female sibling survival) is less than the doubling of daughter numbers present in parthenogenesis induction. Male killers, therefore, reach lower prevalence for a given level of transmission efficiency, and this will lower their probability of causing population damage. It is notable in this context that there are cases of male killers that have invaded a population and settled at relatively low equilibrium prevalence without the evolution of resistance genes and that hosts with male-killer prevalence in the range of 60 to 90% are rather rare. Second, selection at the point of invasion for resistance to the action or transmission of a male killer is higher than for an organism that induces parthenogenesis. A female host infected with a parthenogenesis inducer is fully viable and produces around the same number of progeny as uninfected females, while a male-killer-infected individual produces around 50% the number of progeny of an uninfected individual, even in the absence of direct costs on fecundity. Thus, selection for resistance against parthenogenesis inducers will evolve more slowly, relying solely on Fisherian benefits for producing males in populations made female-biased following the spread of sex-ratio distorters. This would increase the likelihood that a male killer with strong drive would remain polymorphic in the population.

It is easier to examine whether the shortage of males is limiting the population size in extant male killers. The majority of male killers tend to be found in fewer than 50% of females, and because males can usually fertilize far in excess of two females, there will be little risk of population extinction. However, there are a few exceptional cases where the prevalence of the male killer is so high that there are significant numbers of unmated females present. However, even where female reproduction is limited by the shortage of males, the population size may not be greatly reduced. This is because the male killer will also both increase the number of females and reduce levels of competition between males and females, and both of these effects will tend to oppose population extinction due to unfertilized eggs. In a survey of insects, the rate of population growth in the absence of density dependence, R_o, was generally observed to lie between 1.3 and 13 (with some species with higher values) (Hassell et al., 1976). For the common case, where R_o is greater than 2, a male killer will significantly reduce population viability only when female fertility is reduced by one half or more. Given the capacity of males to mate multiply, this will require an extremely high male-killer prevalence.

Sex-Biased Populations and Sexual Selection

In most animals, males have the potential to reproduce faster than females, which has led to the evolution of males that compete for access to females and females that may choose between alternative males (Bateman, 1948). The direction and intensity of this sexual selection depends on the operational sex ratio, which is the ratio of males to females in the population that are available to mate (Emlen and Oring, 1977). If male-killing bacteria bias the population sex ratio toward females, this will also make the operational sex ratio more female-biased. In turn, this is expected to cause a reduction in both the choosiness of females and intensity of competition among males. In extreme cases, it could potentially result in a reversal of sexual selection, with choosy males and competing females. The bacterial prevalence required to reverse the sex roles would depend on the potential rates at which males and females could reproduce given free access to the opposite sex.

The impact of male-killing bacteria on sexual selection has been most comprehensively studied in *A. encedon* (Jiggins et al., 2000c). These effects are likely to be particularly marked in this species — in addition to the populations being very female-biased, the life span and mating rate of males are limited. In southern Uganda, the bacterial prevalence varies over a few kilometers from less than 80% to more than 97% of females being infected. This variation was exploited to investigate how the mating behavior of the butterflies changes with the population sex ratio. In the most female-biased populations, virgin females aggregate at hilltops or other landmarks in large swarms where they vigorously chase other butterflies and exhibit typical butterfly "mate-acceptance" behavior. Once females have mated, they leave the swarm. Similarly, males released in the swarm rapidly mate and then leave the site. This behavior strongly suggests that females are competing for males. Moreover, the swarming of females resembles a common male behavior in insects, where males form swarms in which they compete for females.

Therefore, there is strong evidence that the male-killing infection results in large numbers of unmated females in the population competing for access to males. It is, however, unclear if there has been any evolutionary change in response to the population sex ratio or whether we are observing a behavioral response by the butterflies to the shortage of males. It is also unknown whether male killers have directly influenced the choice of mates made by males in natural populations. Theoretical studies suggest that selection will favor males that choose to mate with females that do not carry the infection (Randerson et al., 2000b), but field studies of *A. encedon* have failed to provide any support for this hypothesis.

This example is clearly atypical of male killers, which typically cause far smaller shifts in the population sex ratio and in which such dramatic reversals of behavior are unlikely. However, we do expect slight shifts in many sexually selected traits such as female choosiness, male–male competition, and sperm allocation in many other species.

Resistance to the Action or Transmission of Male-Killing Bacteria

The fact that male-killing bacteria kill male hosts means that they are always likely to be detrimental to their host, notwithstanding any positive effects of compensation on female host survival or any direct physiological benefit that accrues from bacterial anabolic activity. Selection will therefore favor the spread of host nuclear genes that prevent either the transmission or action of these bacteria.

Resistance genes will spread provided they do not have a cost that exceeds the benefit arising from the production of surviving sons from infected females. Given that the benefit of preventing male-killer action or transmission is positively related to parasite prevalence, while the cost is not, modifiers that prevent the action of male killers that naturally exist at low prevalence will spread only if they have low cost, while modifiers that prevent the action of male killers that naturally exist at high prevalence may have higher costs.

Resistance genes that invade a population initially will go to fixation if uninfected individuals with the resistance genes have the same fitness as those without (i.e., the modifier is cost free). If the modifier has a cost, however, it may arrive at a polymorphic equilibrium in the population. In

the former case, the male-killing bacterium is likely to become lost, unless either the host resistance gene in question is of low efficiency or if counteradaptation occurs on the part of the bacterium. It has been noted that the presence of resistance genes can, in theory, stabilize multiple male killers within a population (Randerson et al., 2000a). However, the parameter space in which multiple infections are maintained is small, and it is questionable whether resistance genes really do represent the factor stabilizing multiple male-killer systems. The coexistence of multiple symbionts may in fact indicate some other incorrect assumption within current models. For example, as yet we do not have the data to determine whether any male killer is maintained at a stable equilibrium for a significant period of time.

The population genetic theory relating to the spread of resistance to male-killing bacteria is currently much better developed than empirical studies of resistance in natural populations. What is established is that polymorphism for resistance to transmission of male killers is known in some systems but does not exist in others. Resistance to the transmission of *S. poulsonii* has been observed in *D. willistoni*. Malogolowkin (1958) observed that certain strains of *D. willistoni* were refractory to *Spiroplasma* transmission. Further, resistance to the transmission of the unknown male killer of *D. prosaltans* has been recorded, with host resistance being explained by a single gene and the resistant allele being recessive (Cavalcanti et al., 1957). However, searches of *D. bifasciata* lines for resistance to male-killer transmission or action proved fruitless; the male-killing *Wolbachia* was transmitted and killed males perfectly in all 38 lines tested (Hurst et al., 2001).

We can therefore state that resistance to the transmission of male-killing bacteria can but does not necessarily evolve. This conclusion parallels the more extensive research that has been undertaken for other selfish genetic elements. The genetic mechanism underlying resistance is completely unknown, and it would seem that knowledge of it is some way off. Parallels with other host–parasite interactions (Carius et al., 2001) suggest that we may need to look beyond a host genotype that resists the transmission of all parasite strains to a host genotype × parasite genotype interaction. There is some suggestion for such an interaction in *D. willistoni*, where the cost of infection shows strong host × parasite interaction effects (Ebbert, 1991). Parallels with other systems also suggest that resistant genotypes may act at the point where the parasite crosses epithelia, for example, into the developing egg. It is notable that *S. poulsonii* fails in other species of fly not through inability to live in the host but from an inability to be vertically transmitted into the eggs. However, one area in which these interactions are likely to differ from other parasite–host associations is in the role of the innate immune system. While these systems are enabled and function effectively against bacteria that are injected into an insect, studies in *Drosophila* indicate they are not induced against inherited bacteria (Bourtzis et al., 2000). Resistance is likely to involve different mechanisms and produce new insights into wholly novel pathways of immunity in insects.

The study of resistance to the action of these agents is even less developed but potentially as interesting. Resistance to the action of male-killing bacteria, genes that prevent infected males from dying, could take two forms. First, the genes could repress the killing mechanism of the bacterium. Second, the genes could represent alterations in the sex-determination system of the host, preventing the bacterium from cueing in on host sex. Male killers must interact with the sex-determination system at some level, and the ability of some male killers to act early in embryogenesis suggests that the elements of host sex used are early in the sex-determination cascade and are not peripheral late-acting parts of sex determination (Hurst and Jiggins, 2000).

The interaction of male-killing bacteria with the host's sex-determination system produces the tempting hypothesis that these elements may actually drive the evolution of these systems in insects. Insect sex determination shows wide diversification, with the genes involved changing despite their function remaining constant. Mutations in sex-determination pathways are usually highly deleterious, so how could a novel system ever have spread? Male-killing bacteria represent a potent selective force in this context. If a population has a male killer at high prevalence, then mutations within the sex-determination pathway that lower the fitness of uninfected males or females (producing "bad" sex determination) may spread if they "save" infected males from death. Just as feminizing

bacteria can induce the spread of novel sex-determining genes, so might male killers. It is notable here that male killers have a higher cross-species incidence than feminizers, potentially making them a more widely important agent in driving the evolution of sex-determining systems. This line of thought is exciting but clearly begs empirical work. How do male killers detect host sex? Can we find natural variation in host resistance to the action of male-killing agents? There is suggestive evidence, with reports of loss and recovery of male killing within lineages, but more data are needed.

In addition to alterations in sex-determination systems, male killers may also facilitate the evolution of parthenogenetic reproduction. The initial mutation producing parthenogenetic reproduction is usually very deleterious, and although spontaneous occasional parthenogenetic reproduction is fairly common across insect taxa, it is usually very inefficient and is more of a curiosity than an adaptive feature. However, in a population where male-killing bacteria are at high prevalence, and females suffer a cost from forced virginity or lack of sperm, the production, even inefficient, of parthenogenetic progeny may become adaptive. It is most likely to spread if it is conditional on lack of sperm, that is to say, sexual reproduction is used if sperm are available.

SUMMARY

It is now established that male-killing bacteria occur in a wide range of arthropods. Late male killing was until recently thought of as being associated with aquatic insects, but the recent discovery in a moth suggests many more cases may be revealed in terrestrial species. Early male killing has long been noted as a phenomenon observed in a wide array of hosts and is now recognized as being phylogenetically widespread within bacteria: if a group of bacteria is vertically transmitted in insects, the presence of a male-killing strain in that group is likely.

We now understand the population biology of male-killing bacteria in its most rudimentary form. The pace and precision of theoretical work has, as ever, outstripped empirical work, and empirical case studies are now beginning to challenge our understanding of male-killer population biology gained from modeling and are refining our understanding. The high prevalence achieved by male killers in some species despite inefficient vertical transmission and the coexistence of different male-killing strains within a single population can both be explained on current models but stretch the assumptions to such a point that the alternative hypothesis — that current understanding is missing some aspect of their population biology — is becoming increasingly tenable to a pragmatic observer. It would certainly seem premature to say we understand the population biology of these elements, and deficiencies in our ability to measure certain parameters are preventing a total understanding of why prevalence varies among associations.

Finally, the high prevalence achieved by male killers in certain species makes them tempting prospects for driving important evolutionary changes. Host extinction may happen but is perhaps unlikely to be common. Alterations in the behavioral ecology of reproduction are likely but may be considered of narrow import, given the general lability of this kind of trait over evolutionary time. Perhaps the most important changes are those that would usually be too costly to spread — modifications in sex-determination system. Parthenogenesis may equally spread and then become refined more easily within a species where male killers are common. Here is a big idea, but the need for definitive case studies remains.

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