HOW CAN SEX RATIO DISTORTERS REACH EXTREME PREVALENCES? MALE-KILLING *WOLBACHIA* ARE NOT SUPPRESSED AND HAVE NEAR-PERFECT VERTICAL TRANSMISSION EFFICIENCY IN *ACRAEA ENCEDON*

FRANCIS M. JIGGINS,[1,2] JAMES P. RANDERSON,[3] GREGORY D. D. HURST,[4] AND MICHAEL E. N. MAJERUS[1]
[1]*Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, United Kingdom*
[2]*E-mail: fmj1001@mole.bio.cam.ac.uk*
[3]*Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom*
[4]*Department of Biology, University College London, 4 Stephenson Way, London NW1 2HE, United Kingdom*

Abstract.—Maternally transmitted bacteria that kill male hosts early in their development are found in many insects. These parasites typically infect 1–30% of wild females, but in a few species of insects, prevalences exceed 95%. We investigated one such case in the butterfly *Acraea encedon*, which is infected with a male-killing *Wolbachia* bacterium. We measured three key parameters that affect the prevalence of the parasite: transmission efficiency, rate of survival of infected males, and the direct cost of infection. We observed that all wild females transmit the bacterium to all their offspring and that all infected males die in wild populations. We were unable to detect any physiological cost to infection in lab culture. These observations explain the high prevalence of the *A. encedon* male killer, as theory predicts that under these conditions the parasite will spread to fixation. This will occur provided the death of males provides some benefit to the surviving infected females. The problem therefore becomes why the bacterium has not reached fixation and driven the butterfly extinct due to the shortage of males. We therefore investigated whether males choose to mate with uninfected rather than infected females, as this would prevent the bacterium from reaching fixation. We tested this hypothesis in the ''lekking swarms'' of virgin females found in the most female-biased populations, and were unable to detect any evidence of mate choice. In conclusion, this male killer has spread to high prevalence because it has a high transmission efficiency and low cost, but the factors maintaining uninfected females in the population remain unknown.

Key words.—*Acraea encedon*, male killer, mate choice, paradox of the lek, population dynamics, *Wolbachia*.

Received May 27, 2002. Accepted July 19, 2002.

Many animals are host to maternally transmitted bacterial symbionts. These symbionts are selected to distort the host sex ratio towards daughters, because only females will pass them to the next generation (Cosmides and Tooby 1981). One of the most common mechanisms of sex ratio distortion is to kill males early in embryonic development. In hosts with sibling competition or cannibalism, this increases the survival or fecundity of the surviving females, and therefore allows the bacterium to spread through the host population (Hurst 1991; Hurst and Majerus 1993).

The population dynamics of male-killing bacteria are well characterized theoretically, and three major parameters are thought to determine whether a male killer will invade a population and what its equilibrium prevalence will be (Hurst 1991). The first is the amount by which the fitness of females is increased when their brothers die, which is dependent on ecological factors such as the rate of sibling competition, inbreeding, and sibling cannibalism. The second parameter is the efficiency with which infected females transmit the bacterium to their offspring. The transmission efficiency may be affected by host effects such as any immune response, bacterial characteristics, and environmental factors such as temperature. Finally, infection with a male killer may carry some physiological cost for the host, which will reduce the prevalence of the infection. Alternatively, it has been suggested that some male killers may have evolved to physiologically benefit female hosts, which will increase the prevalence (Jiggins et al. 2000a).

This basic set of three parameters is expected to affect the population dynamics of all male killers. Theoretical studies have also identified a number of secondary factors that may

be important in certain species. First, male killer prevalence will be reduced if some infected males survive. This could occur due to environmental factors (Hurst et al. 2000), or due to host genes suppressing the male-killing phenotype (Randerson et al. 2000a). Second, if males choose to mate with uninfected females, this could also reduce the prevalence of infection (Randerson et al. 2000b).

Despite this rich theoretical background, there is scant data on the parameters in these models. A particular problem is that these measurements should ideally be made in the field because they are dependent on environmental and ecological conditions. For example, the transmission efficiency of *Wolbachia* bacteria varies greatly between laboratory cultures and natural populations (Turelli and Hoffmann 1995).

The best-described parameter is the prevalence of infection. Typically 1–30% of wild females carry the male killer, but there are a few exceptional cases in which more than 95% of females are infected (Clarke et al. 1983; Jiggins et al. 2000a,b). Here we ask why these extreme prevalences occur, and what prevents the male killer reaching fixation and driving the host extinct. We measured most of the parameters described above in the butterfly *Acraea encedon*, which is host to male-killing *Wolbachia* bacterium that infects over 95% of females in some populations (Jiggins et al. 1998; Hurst et al. 1999; Jiggins et al. 2000b).

The aims of this study were to use a natural population to (1) measure the transmission efficiency of the bacterium in wild females, (2) test for the presence of suppression of the male-killing phenotype by looking for infected males, (3) test whether uninfected females have a mating advantage within a population, and (4) measure the physiological costs or benefits associated with male killer infection.

Materials and Methods

Transmission Efficiency and Suppression of Male Killing

We estimated the efficiency with which a male killer transmits from an infected female to its offspring from the sex ratio produced by infected females, as males that do not inherit the bacterium will survive (Jiggins et al. 2000a). Similarly, if the male-killing phenotype of the bacteria is being suppressed by the host, this will result in the survival of infected males. It is important to measure these parameters in wild females, as it is known that they may differ from measurements taken using laboratory cultures (Turelli and Hoffmann 1995).

Wild female *A. encedon* were collected from the Entebbe botanic gardens population in southern Uganda, which is described by Jiggins et al. (2000b). The females were allowed to oviposit on the host plant *Commelina benghalensis*, and a piece of abdomen tissue was stored in 100% ethanol at 4°C. This tissue was tested for *Wolbachia* following the protocol described in Jiggins et al. (2000b). In brief, DNA was extracted using a chelex-based method, and tested for infection using diagnostic polymerase chain reaction (primers *wsp*81F and *wsp*691R), which amplifies the *wsp* surface protein gene of *Wolbachia* (Zhou et al. 1998). Negative results were replicated twice to confirm the uninfected status of the individuals. Insect specific primers that amplify the ITS1 region (4S and BD1) were used to check that the DNA extraction process had been successful (Hillis and Dixon 1991).

The eggs laid by females were reared to adulthood in either Uganda or Cambridge, United Kingdom, on *C. benghalensis*, and the sex ratio was recorded. In addition, the parents of most of the F1 broods reported in two previous studies (Jiggins et al. 1998, 2001) were tested for *Wolbachia*, and infected lines were added to the dataset. Rearing conditions in these previous studies were similar to those used in the current one.

The approach outlined above will detect both imperfect transmission of the bacterium, and survival of infected males. We also collected wild males from the same populations and tested them for the presence of *Wolbachia*, as an additional method to detect the survival of infected males.

Physiological Cost of Infection

The second aim of this study was to measure any physiological cost of infection. We collected wild females and allowed them to lay eggs. Forty eggs from each clutch were carefully removed from the leaf and spaced out in a petri dish. This prevented hatchlings coming into contact with each other or unhatched eggs, and so controlled for the effect of male death on the fitness of females by separating female and male eggs. On the day of hatching we set up pairs of infected and uninfected egg clutches (initially these were identified from the egg hatch rate and later confirmed by PCR). Immediately on hatching the infected and uninfected larvae from each pair were pooled on the same leaf of a single plant (i.e., about 20 infected female larvae and 40 uninfected larvae of both sexes). They were then reared mixed together on an excess of food, and the date of emergence and dry mass of newly emerged adults recorded. Finally, the infected

and uninfected females were identified by post hoc PCR. This method ensured that infected and uninfected larvae were subject to identical conditions, and allowed us to compare larval mortality, development time, and adult mass. The comparisons of larval mortality assumed the primary sex ratio of eggs laid by infected and uninfected females was the same and that all the offspring of infected females inherited the bacterium (see Results).

Mating Advantage of Uninfecteds and Mate Choice

Our final aim was to test whether uninfected females are more likely to mate than infected ones. This could result from male mate choice, or from uninfected females being better at competing for mates. Both hypotheses are plausible because, in the most female-biased populations, females form swarms in which they compete for males (Jiggins et al. 2000b). To test this hypothesis, males were released into the swarm, and the infection status of the females they mated with recorded.

The experiment was carried out at Makerere and Ggangu, near Kampala in Uganda. These are both hilltop sites at which the butterflies form a ''lekking swarm'' in the afternoons, and are described by Jiggins et al. (2000b). Initially, on successive afternoons, every effort was made to catch all *A. encedon* at the hilltops. These samples were used to estimate the prevalence of the *Wolbachia* bacterium in the populations prior to release experiments. The daily intensive sampling had only a small effect on the numbers of *A. encedon* congregating at the sites. Any mating pairs were recorded as such, and were regarded as ''natural choices.'' A further, smaller sample was taken in the same way following release experiments, in order to check whether the bacterial prevalence had changed during the experiment.

The males for release were from laboratory-reared lines collected previously at Entebbe. After emergence they were kept in a large outdoor cage with females from the same lines. The males consequently had mated prior to their use in a release experiment. On the day of an experiment, males were fed on sugar solution and transported in paper envelopes to the field site. They were then released individually at the focal point of the swarm (i.e., where there was the highest concentration of females) by placing them on vegetation. Behavioral data were recorded for a subset of the released males.

Following their release, the males were followed continuously within the swarm until they either mated or left the swarm without mating. Once the abdomens of the male and female were joined, the pair was left for several minutes to ensure that this was a genuine mating. Because the process of mating in these butterflies takes an entire day, we are confident that the released butterflies were not mating other females without us noticing.

The infection status of both the ''chosen'' females and the controls collected before and after the experiment was determined as described above. The statistical analysis of these results primarily consisted of testing 2×2 contingency tables for independence. The appropriate test given our experimental design is the G test (Sokal and Rohlf 1995). However, this test becomes inaccurate at small sample sizes (Sokal and

TABLE 1. Mean development time, mean dry mass, and survival of infected and uninfected female *Acraea encedon*. The proportion of female zygotes surviving to adulthood assumes that exactly 50% of the eggs in the clutch were female. In each pair the larger of the two values is in bold.

Pair	Mean dry mass (g)		Mean development time (days)		Female survival	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
1	0.046	**0.049**	45.5	**46.0**	0.88	**0.98**
2	0.031	**0.036**	51.9	51.7	0.67	**1.00**
3	**0.039**	0.035	**48.6**	48.3	**0.87**	0.56
4	**0.041**	0.034	47.1	**48.2**	**0.85**	0.80
5	**0.039**	0.030	53.4	**54.8**	**1.15**	0.95
6	**0.040**	0.036	**55.3**	52.0	**0.80**	0.77
7	0.041	**0.042**	**46.5**	45.8	**0.80**	0.75
8	**0.037**	0.036	46.9	**49.3**	**0.90**	0.75
9	**0.039**	0.033	**64.1**	63.5	0.65	**0.75**

TABLE 2. Analysis of variance on adult dry mass.

	Sum squares	Mean square	df	*F*-ratio	*P*
Pair	0.0052	0.0007	8	3.07	0.07
Wolbachia	0.0005	0.0005	1	2.50	0.15
Pair × infection	0.0017	0.0002	8	5.87	<0.0001

Rohlf 1995), and when any of the expected values in the contingency table fell below five we used Monte Carlo simulation (50,000 replicates) in place of the G test (Lewontin and Felsenstein 1965).

RESULTS

Transmission Efficiency and Suppression of Male Killing

A total of 2879 females and no males were reared from 102 infected female *A. encedon* (mean brood size = 28). Previous work has shown that *Wolbachia*-infected females can be heterogeneous in their transmission efficiency, with a small fraction of females accounting for most of the uninfected offspring produced (Turelli and Hoffmann 1995; Jiggins et al. 2000a). Therefore, it is conservative to take the sample size as 102, giving a 95% confidence interval of 96–100% of females producing only daughters.

The transmission efficiency of *Wolbachia* in *A. encedana*, a closely related butterfly and bacterium, has been estimated previously. In *A. encedana*, three of 37 females showed imperfect transmission, passing the bacterium to only about 50% of their offspring (Jiggins et al. 2000a). This is a significantly higher proportion of females showing imperfect transmission than we observed in *A. encedon* (Monte Carlo simulation, 50,000 replicates; *P* = 0.02).

A total of 114 wild males were tested for *Wolbachia*, and all proved negative. This result and the lack of males in the breeding data suggest that no infected males survive. From this, we can conclude that there is no suppression of the male-killing action of the bacterium by the host.

Cost of Infection

In total, we set up nine pairs of infected and uninfected females, with each pair consisting of 40 infected and 40 uninfected eggs pooled on a plant (720 eggs in total). These results are summarized in Table 1. In six of nine pairs, the mean dry mass of infected females was higher than that of uninfected ones. However, this difference is not significant, as an analysis of variance (ANOVA), showed there was no effect of infection status on dry mass (Table 2). There was, however, a highly significant interaction between pair and

infection status (Table 2). The most likely explanation of this is that there were genetic differences between host lines.

The data on development time was not normally distributed, and was therefore not analyzed by ANOVA. However, it is clear from Table 1 that there is no consistent effect of infection on development time. In six of nine pairs the infected females developed more slowly than uninfected ones. There was, however, significant variation between the pairs (Kruskal-Wallis test: *P* < 0.001).

Finally, we looked at the rate of mortality, and again there was no effect of infection. In six of nine comparisons, infected females had lower rates of mortality (Table 1). Therefore, we were unable to detect any effect of *Wolbachia* on any of the fitness-related traits that we measured.

Behavioral Data

In order to assess the plausibility of males choosing uninfected females, we observed the behavior of males in the sex-role-reversed swarms. Behavioral data were recorded for a subset (*n* = 21) of the released males, and the statistics that follow are intended merely to give an impression of male behavior. After an initial inactive period, most males reacted to females flying overhead by taking off and giving chase. These mid-air chases were usually short (<15 sec). Males that mated took a median of 3 min to do so after their release. Of this, a median of 40 sec was spent in the air and they engaged in a median of four mid-air chases. After a mid-air encounter with a female, some males broke off the chase and landed. Alternatively, some males would begin chasing another female while in mid-air. After a chase in which the male did not switch to a different female or land, the female would land and rest with a ventrally curved abdomen. The male would then land on top of her and position himself for mating. In a number of cases the male appeared to have grasped the female's abdomen with his claspers but subsequently broke off and began flying again. The next phase involved the female taking off with the male attached and transporting him a few meters away from the focus of the lekking swarm.

Some males did not take any interest in the congregating females around them and flew directly upwards into the trees. Although initially lost to the experiment, we often recaptured these males either later the same day or on subsequent days. Almost invariably, these males were *in copula* when recaptured. These pairs were taken to represent genuine choices by males and were used in the analysis below.

Mating Advantage of Uninfecteds and Mate Choice

The observations above show that males interact with multiple females before mating, and therefore have the opportunity to choose between females. Therefore we tested wheth-

TABLE 3. The prevalence of *Wolbachia* and sex ratio of field samples.

Site	Pre-release population sample					Mates of released males			Post-release population sample				
	No. females	No. unin-fected females	% unin-fected females	No. males	% males	No. female partners	No. unin-fected	% unin-fected	No. females	No. unin-fected females	% un-infected females	No. males	% males
Ggangu	134	20	14.9	4	2.9	87	3	3.4	49	6	12.2	1	2.0
Makerere	323	9	2.8	1	0.3	56	1	1.8	87	8	9.2	1	1.1

er uninfected females were chosen. The bacterial prevalence and sex ratio of the butterflies at the hilltop sites is recorded in Table 3. At Ggangu, the data support the notion that bacterial prevalence in females remained constant while release experiments were carried out (14.9% uninfected before, 12.2% after; $G = 0.551$, df $= 1$, $P = 0.46$; Table 3). The population prevalence was therefore taken to be the combination of the before and after sample. However, at Makerere, bacterial prevalence decreased during the course of the release experiment (2.8% uninfected before, 9.2% after; Monte Carlo simulation, $P < 0.05$; Table 3). Thus, when testing whether uninfected females were more likely to mate, the bacterial prevalence in the ''chosen'' females was conservatively compared to the postrelease sample.

A total of 172 males were released: 111 at the Makerere site and 61 at Ggangu. Of these, 144 were recaptured *in copula*. Does the bacterial prevalence in the mating females differ from the bacterial prevalence in the population? Contrary to the hypothesis of male choice, there was a higher proportion of infected females in the chosen sample than in the population as a whole at both of the sites (Table 3). At Ggangu this difference was statistically significant (14.2% of females in the population were uninfected compared to only 3.4% of mating females; $G = 8.49$, df $= 1$, $P < 0.005$). However, at Makerere the prevalence in the chosen females was not significantly different from the population as a whole (9.2% of the postrelease population was uninfected compared to 1.8% of mating females; Monte Carlo simulation, not significant). In addition, we captured three wild pairs *in copula* (two at Makerere and one at Ggangu). In all of these ''natural choices,'' the female was infected.

The trend at both sites, therefore, is for there to be fewer uninfecteds in the mating sample than in the control. A more rigorous test of this hypothesis is possible by combining all the data from the two sites and using a technique developed by Fisher to combine the two *P*-values (Sokal and Rohlf 1995). This reveals a marginally significant tendency for males to choose infected females more often than uninfected females ($P < 0.05$). In generating this probability we conservatively used the prerelease population sample from Makerere. Therefore, we can reject the hypothesis that uninfected females have a mating advantage within the swarms. This data argues strongly against the existence of mate choice in favor of uninfecteds. Thus, the data suggest, if anything, precisely the opposite situation to that predicted by theoretical modeling (Randerson et al. 2000b).

In addition to measuring bacterial prevalence, the mating status of a subset of females in three populations was determined by dissecting their reproductive tracts and recording the presence/absence/number of spermatophores. It is known

that males only transfer one spermatophore per mating (Jiggins et al. 2000b). One check on the reliability of this technique was that all females that subsequently laid viable eggs ($n = 72$) were found to contain at least one spermatophore (dissections were carried out without knowing whether the female had laid viable eggs).

Given that males appear not to be choosing uninfected females, is it possible to reproduce the previously reported finding that uninfected females at the congregations are more likely to have mated (Jiggins et al. 2000b)? First, the proportion of mated females at the congregations was low (Makerere, 11 of 157 females; Ggangu, 17 of 143 females). All females that were mated contained one spermatophore (except one at Ggangu that harbored two). These findings match previous results (Jiggins et al. 2000b) and are to be expected given the scarcity of males at the populations with the bacteria at high frequency.

At Makerere, none of the uninfected females ($n = 8$) contained spermatophores, but among the infected females ($n = 136$), 11 were mated. This difference was not significant (Monte Carlo simulation, not significant). At Ggangu, of 17 uninfecteds, four had mated, compared with 19 of 122 infecteds. Again, these are not significantly different (Monte Carlo simulation, not significant). The data, therefore, do not support the hypothesis that infected females are more likely to be virgins. This runs contrary to data collected previously at the Makerere site (Jiggins et al. 2000b).

This situation contrasts sharply with that at Entebbe, where the butterflies were collected from around the larval food plant rather than from a swarming site. Here the majority of females contained at least one spermatophore (156 of 213 females dissected). Indeed many females here contained multiple spermatophores; 14 females harbored two and one had three spermatophores. However, the likelihood that a female had been mated did not differ between infecteds and uninfecteds (139 of 188 infected females and 17 of 25 uninfected females; $G = 0.38$, df $= 1$; not significant).

DISCUSSION

Transmission Efficiency and Suppression

In *A. encedon* the extreme scarcity of males means that there is strong selection to either prevent transmission of the parasite to the next generation or suppress its phenotype. Remarkably, we were unable to find any evidence that the butterfly has evolved either of these traits. It therefore seems that the parasite has successfully evaded host defenses.

Infected females produced exclusively female offspring, which implies that all the male eggs in our sample inherited the bacterium and were killed. This contrasts with the typical

pattern observed for male-killing bacteria, where a proportion of eggs fails to inherit the parasite (Hurst and Majerus 1993). Therefore, the high transmission efficiency of the bacterium can explain why the prevalence of infection is higher in this system than in most others.

Interestingly, the transmission efficiency is significantly higher than that of a male killer in *A. encedana*, which also infects more than 95% of females (Jiggins et al. 2000a). This is surprising as both the bacterium and butterfly are very closely related to the *A. encedon* system (Jiggins et al. 2000a). In *A. encedana* we calculated that the transmission efficiency of the bacterium was so low that some other factor such as a physiological benefit to infection must account for the high prevalence of infection. However, it seems improbable that the causes of high prevalence differ between such closely related systems, and our earlier conclusion must be treated with some caution.

We were also unable to detect any infected males within the population, as would be expected if there were host genes which suppress the act of male killing or environmental factors that prevent the bacterium killing males. This raises the question why the butterfly has failed to evolve to either prevent transmission of the bacterium or suppress the male-killing phenotype of the parasite. It is possible that *Wolbachia* has evaded host immune defenses by living within host cells (Bourtzis et al. 2000). It may also detect the sex of the host using conserved and essential elements of the sex determination pathway that cannot easily evolve to prevent the bacteria recognizing males.

Theory predicts that a male killer that has perfect vertical transmission and is not suppressed by the host will spread to fixation, driving the host extinct. There are several reasons why this may not have occurred. First, if the benefits to females of their brothers dying are very small, then extremely low levels of imperfect transmission or suppression can maintain a polymorphism, and such effects may go undetected in our sample. Second, the transmission efficiency may vary with temporal or spatial changes in the environment. Therefore, extinction may be prevented because the transmission efficiency is lower either in neighboring populations, or different seasons. Finally, some other form of frequency-dependent selection may act against the bacterium when it is common. One possibility that we tested is that males choose uninfected females.

Mating Advantage of Uninfecteds and Mate Choice

A mating advantage of uninfected butterflies is of interest for three reasons (Randerson et al. 2000b). First, it may maintain a stable polymorphism of infected and uninfected females. Second, it could explain the evolution of sex-role-reversed lekking swarms in *A. encedon*. These are swarms of virgin females in which female butterflies compete for males in the most sex-ratio-biased populations (Jiggins et al. 2000b). Third, theory predicts that selection can maintain a stable polymorphism of the genes determining male choice and a polymorphism of infected and uninfected females. This represents a solution to the 'paradox of the lek,' which arises because mate choice will lead to loss of genetic variation in the trait being chosen.

The data presented here strongly reject the notion that male *A. encedon* choose to mate preferentially with uninfected females. In experiments where captive males were released at female aggregation sites, the batch of females sampled by males in fact had a higher proportion of infecteds than the population as a whole.

We were also unable to reproduce the previous result (Jiggins et al. 2000b) that, in a wild sample, uninfected females were more likely than infected females to have mated. The reason for this difference is a matter of speculation, but it is possible that the earlier result was due to immigration of mated uninfected females from nearby populations. Therefore, this result is compatible with our conclusion that there is no mate choice.

Having established that males are not choosing uninfected mates (and indeed appear to be doing the opposite), we are left with the question of why females form lekking swarms in populations where males are scarce. It is possible that males use the sites to choose some other aspect of female phenotype, but what that might be is not obvious from casual observation. Alternatively, the sites might facilitate the quick location of females by males, or may attract males from more distant populations where the bacterial prevalence is lower. Virgin females attending such sites might be at an advantage if the number of males attracted is disproportionately larger than the number of receptive females at the site. This hypothesis is consistent with the observation that the aggregations are always located at prominent landmarks such as on hilltops and beside tall trees. In many other insects, males aggregate at landmarks, which is thought to aid their location by females (Shields 1967; Alcock 1987). Another possibility is that the aggregations reduce predation on the butterflies. Warning-colored, distasteful insects are often thought to aggregate in order to reduce predation by 'naïve' predators (Gagliardo and Guilford 1993).

In other *Acraea* species, hilltopping behavior (small congregations, typically of males) is widespread (Jiggins 2002). In *A. encedon*, males and females might search for mates at grassy areas near trees in all populations (whether *Wolbachia* is at high or low frequency). If pairs leave the sites once they have started mating (as we observed), then in a population with a 1:1 sex ratio, the numbers of butterflies at the congregation site will be insignificant at any one time. Thus, a 'lekking swarm' might not be recognized as such. Conversely, in populations with the infection at high prevalence (and hence with scarce males), there will always be a large number of unmated females at the site who have failed to find a partner. This hypothesis would explain both the absence of congregations in populations with low infection frequency, and the previous result that unmated females are more likely to remain at mating congregations (Jiggins et al. 2000b).

Cost of Infection

Vertically transmitted parasites rely on the survival and reproduction of their hosts to be transmitted. Therefore, they are selected to have low virulence in the transmitting sex. This appears to be the case in *A. encedon*, as we were unable to detect any effect of *Wolbachia* on survival, development time, and adult size of female butterflies. It is possible that

Wolbachia does affect some other component of female fitness such as fecundity, fertility, age of first reproduction, or adult longevity. Despite these limitations to our experiments, these effects would not maintain uninfected females in the population unless their magnitude depends on the frequency of infection.

Population Dynamics of Male Killers

A male-killing bacterium can invade a population if the death of males benefits the surviving females sufficiently to counteract the loss of infection by imperfect transmission and any physiological cost to the host. This is reflected in the observation that male killers are typically observed in species with strong antagonistic interactions, such as cannibalism, between siblings (Hurst and Majerus 1993). However, if the male killer has perfect or near-perfect vertical transmission efficiency, and imposes little or no cost on the host, then it can still invade and spread to high prevalence even if the benefit to females of male death is extremely small.

In conclusion, our observations suggest the low cost and high transmission efficiency of the bacterium explain its high prevalence. Furthermore, it is possible that any benefits to females from the death of their brothers may be very small. This is in contrast to observations of other male killers found at lower prevalence that typically have both imperfect transmission, and, in the few cases studied, impose a physiological cost on the host (Hurst et al. 1997).

Literature Cited

Alcock, J. 1987. Leks and hilltopping in insects. J. Nat. Hist. 21: 319–328.

Bourtzis, K., M. M. Pettigrew, and S. L. O'Neill. 2000. *Wolbachia* neither suppresses nor induces antimicrobial peptides. Insect Mol. Biol. 9:635–639.

Clarke, C., G. Johnston, and B. Johnston. 1983. All female broods in *Hypolimnas bolina* (L.). A resurvey of West Fiji after 60 years. Biol. J. Linn. Soc. 19:221–235.

Cosmides, L. M., and J. Tooby. 1981. Cytoplasmic inheritance and intragenomic conflict. J. Theor. Biol. 89:83–129.

Gagliardo, A., and T. Guilford. 1993. Why do warning-colored prey live gregariously? Proc. R. Soc. Lond. B 251:69–74.

Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66:411–453.

Hurst, G. D. D., and M. E. N. Majerus. 1993. Why do maternally inherited microorganisms kill males? Heredity 71:81–95.

Hurst, G. D. D., L. D. Hurst, and M. E. N. Majerus. 1997. Cytoplasmic sex ratio distorters. Pp. 125–154 *in* S. L. O'Neill, A. A. Hoffman, and J. H. Werren, eds. Influential passengers: microbes and invertebrate reproduction. Oxford University Press, Oxford, U.K.

Hurst, G. D. D., F. M. Jiggins, J. H. G. Schulenberg, D. Bertrand, S. A. West, I. I. Goriacheva, I. A. Zakharov, J. H. Werren, R. Stouthamer, and M. E. N. Majerus. 1999. Male-killing *Wolbachia* in two species of insect. Proc. R. Soc. Lond. B 266:735–740.

Hurst, G., A. Johnson, J. Schulenburg, and Y. Fuyama. 2000. Male-killing *Wolbachia* in *Drosophila*: a temperature sensitive trait with a threshold density. Genetics 156:699–709.

Hurst, L. D. 1991. The incidences and evolution of cytoplasmic male killers. Proc. R. Soc. Lond. B 244:91–99.

Jiggins, F. M. 2002. Widespread 'hilltopping' in *Acraea* butterflies and the origin of sex-role-reversed swarming in *Acraea encedon* and *A. encedana*. Afr. J. Ecol. 40:228–231.

Jiggins, F. M., G. D. D. Hurst, and M. E. N. Majerus. 1998. Sex ratio distortion in *Acraea encedon* (Lepidoptera: Nymphalidae) is caused by a male-killing bacterium. Heredity 81:87–91.

Jiggins, F. M., G. D. D. Hurst, C. E. Dolman, and M. E. N. Majerus. 2000a. High prevalence male-killing *Wolbachia* in the butterfly *Acraea encedana*. J. Evol. Biol. 13:495–501.

Jiggins, F. M., G. D. D. Hurst, and M. E. N. Majerus. 2000b. Sex ratio distorting *Wolbachia* cause sex role reversal in their butterfly hosts. Proc. R. Soc. Lond. B 267:69–73.

Jiggins, F. M., G. D. D. Hurst, J. H. G. vander Schulenburg, and M. E. N. Majerus. 2001. Two male-killing *Wolbachia* strains coexist within a population of the butterfly *Acraea encedon*. Heredity 86:161–166.

Lewontin, R. C., and J. Felsenstein. 1965. The robustness of homogeneity tests in $2 \times n$ tables. Biometrics 21:19–33.

Randerson, J. P., N. G. C. Smith, and L. D. Hurst. 2000a. The evolutionary dynamics of male-killers and their hosts. Heredity 84:152–160.

Randerson, J. P., F. M. Jiggins, and L. D. Hurst. 2000b. Male-killing can select for male mate choice: a novel solution to the paradox of the lek. Proc. R. Soc. Lond. B 267:867–874.

Shields, O. 1967. Hilltopping. J. Res. Lepid. 6:69–178.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. W. H. Freeman, New York.

Turelli, M., and A. A. Hoffmann. 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. Genetics 140:1319–1338.

Zhou, W. F., F. Rousset, and S. O'Neill. 1998. Phylogeny and PCR based classification of *Wolbachia* strains using *wsp* gene sequences. Proc. R. Soc. Lond. B 265:509–515.

Corresponding Editor: S. Pitnick