ANIMAL BEHAVIOUR, 2002, **63**, 131–141
doi:10.1006/anbe.2001.1877, available online at http://www.idealibrary.com on IDEAL®

Lack of parasite-mediated sexual selection in a ladybird/sexually transmitted disease system

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*(Received 10 April 2001; initial acceptance 12 June 2001;
final acceptance 23 August 2001; MS. number: 6905)*

Despite the clear potential of sexually transmitted diseases (STDs) to affect host mating behaviour via both host and parasite evolution, there have been few explicit tests of the relationships between STDs and sexual behaviour in animals. We investigated the effect of infection on host sexual behaviour within an invertebrate system. *Coccipolipus hippodamiae* is a sexually transmitted ectoparasite of *Adalia bipunctata*, the two-spot ladybird. The parasite feeds on host haemolymph, develops rapidly, and is deleterious to *A. bipunctata* hosts of both sexes. We examined whether infection affected mating success, and whether males or females showed any preferences with respect to infection status. We observed field mating rates of infected and uninfected ladybirds and carried out controlled laboratory experiments. We did not detect any negative effects of parasite infection on host mating vigour, nor any evidence for the existence of a host mating preference based on infection status. In addition, there was no evidence of parasite-induced changes in behaviour, such as increased promiscuity, which would increase transmission opportunities for the parasite. In summary, contrary to a body of speculation, there was no evidence of any connection between infection and mating rate, in either sex. We discuss possible explanations for these findings.

Sexually transmitted diseases (STDs) frequently have highly deleterious effects on their hosts (Lockhart et al. 1996). Thus, STD presence makes sex risky and the hazard of contracting STDs may influence the evolution of mating systems in animal host populations (Hamilton 1990; Sheldon 1993; Loehle 1995). Presence of an STD is likely to lead to adaptations that decrease disease exposure, such as monogamy and mate choice.

Preferential mating with uninfected partners is likely to be favoured in both sexes to avoid infection (parasite transmission avoidance model; Borgia & Collis 1989; Clayton 1991a, b). Differentiation between partners may be on the basis of direct evidence of infection, or the magnitude of showy male traits, if the ability to produce such signals covaries with infection status (Able 1996). Parasite-mediated intrasexual selection might also be expected in the presence of an STD (or any other disease), as infection may weaken males and reduce their ability to compete for mates.

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Consideration of host evolution leads one to predict reduced mating success for infected individuals. In contrast, examination of selection pressures on parasites suggests the opposite. Parasites generally harm the host, if only through use of host resources. However, the pattern of virulence varies. One would expect sexually transmitted parasites not to reduce host mating success and to have minimal effects on longevity, and instead to have virulence effects on host fertility (Knell 1999). This pattern allows parasite reproduction without limiting opportunities for transmission. Sexually transmitted parasites may even manipulate host libido or attractiveness to increase transmission opportunities (Dawkins 1982; Lockhart et al. 1996).

Despite the potential of STDs to affect host mating behaviour via either host or parasite evolution, there have been few tests of the relationships between STDs and sexual behaviour in animals. Only two systems have been studied to date. Female laboratory mice, *Mus musculus*, are less attracted to the odours of males infected with directly (but not exclusively sexually) transmitted parasites (see Kavaliers et al. 2000 for a review), which is consistent with parasite transmission avoidance. There is also evidence compatible with adaptive parasite manipulation of host behaviour from the same system. Males infected with *Eimeria vermiformis* show augmented sexual

behaviour when infective. The second system is an invertebrate host, the milkweed leaf beetle, *Labidomera clivicollis*, and a sexually transmitted parasitic mite. Here, there is no evidence for mate choice on the basis of infection status (Abbot & Dill 2001). However, there is an effect on intrasexual selection. Infected males show increased aggression and better competitive ability than uninfected males, which Abbot & Dill (2001) interpreted as host adaptation, that is, increased allocation to reproduction to compensate for the effects of infection on survival, rather than parasite manipulation.

We used a similar system to test for both positive and negative effects of STD infection on male and female mating success. The host in this case is *Adalia bipunctata*, the two-spot ladybird, which differs from *L. clivicollis* in showing less direct male–male competition and hence a greater opportunity for mate choice. Mating preferences have previously been shown in *A. bipunctata* females for melanic (Majerus et al. 1982) and better-fed partners (Majerus 1994a). The parasite is a sexually transmitted subelytral mite, *Coccipolipus hippodamiae*. Larval *C. hippodamiae* may pass both from male to female, and from female to male, during host copulation. Once transferred, the larvae embed their mouthparts into the underside of the host elytra, feed on host haemolymph and metamorphose into adults. The mite makes heavy demands on host resources, developing quickly on hosts of both sexes, such that within a few weeks the entire underside of the elytra is covered by a mite colony (K. M. Webberley, personal observation). Infection reduces survivorship during overwintering, especially in males (K. M. Webberley & G. D. D. Hurst, unpublished data), and both fertility and fecundity in females (Hurst et al. 1995).

We used two approaches to test comprehensively for any parasite-mediated effects on mating success. First, we used field observations to look for an association between infection status and mating status in the natural population. Field studies can reveal large effects of infection on mating success and allow detection of ecology-dependent intrasexual effects. Second, we undertook laboratory experiments with the aim of detecting more subtle effects and to remove possible confounding effects of host age. The experiments were paired one-on-one mating tests in which we placed individuals with members of the opposite sex that were either infected or uninfected and observed the responses of both parties. Such tests reflect the natural conditions under which *A. bipunctata* males encounter females in the field, that is, sequentially as they bump into them at feeding sites (see Bateman et al. 2001 for a discussion of the resemblance of simultaneous and sequential choice tests to natural systems) and also allow differentiation between the effects of intersexual and intrasexual selection.

METHODS

Field Mating Rates

Sampling and checking infection status

We collected samples of *A. bipunctata* adults by eye from *Rosa* spp. or by beating *Tilia* spp. in Torun, Poland in 1997 and 1999. All were individually placed in Eppendorf tubes marked with their mating status at time of collection (either mating or single), and then scored for sex on the basis of abdominal characteristics (see Randall et al. 1992 for methodology) and for the presence or absence of *C. hippodamiae*. To score infection status we used methodology similar to that of Hurst et al. (1995). After making the ladybirds inactive by chilling in a refrigerator we used a paintbrush to fix them on their backs in Blue-tac. Whilst viewing through a microscope, we exposed the undersides of the elytra by manipulating the abdomen away from the elytra with entomological pins, thus revealing any infection.

In 1997, we collected 124 ladybirds over 6 days between 19 June and 4 July. In 1999, we collected five large weekly samples of *A. bipunctata* between 25 April and 24 May, producing a total sample size of 596. The ladybirds scored were all from the overwintered generation (one individual from the emerging cohort was collected, but was excluded to avoid the confounding effects of age). To make sampling unbiased with respect to mating and infection status, we took care to collect all the ladybirds from each individual rose bush and all the ladybirds from the lower branches of each tree collected from.

Analysis

The null hypothesis that mating status is independent of infection status was tested with two-tailed χ^2 and Fisher exact test analysis. We analysed the data from the different years and different sexes separately.

Laboratory Experiments

Materials

We used 22 full-sib lines of *A. bipunctata* in the two laboratory experiments. The experimental beetles were the F_2 generation of beetles collected from field sites at which *C. hippodamiae* was present at high prevalence. The pairs of males used in each replicate of the experiments were brothers, reared together from the same egg clutch. Similarly, within each replicate, the pairs of females were same-cohort, full-sibling sisters, unrelated to the male. The ladybirds were reared at room temperature under constant light at low density, 10 larvae per 9-cm-diameter petri dish, with the dishes changed every 2 days to prevent diseases. They were fed excess high-quality food, the pea aphid, *Acyrthosiphon pisum*. The two sexes were kept separately from 3 days after eclosion so that mating experience could be controlled. Within sibling pairs, males and females were matched for colour pattern and mass to within 0.5 mg to control against any potentially confounding effects of these factors (Muggleton 1979; O'Donald & Muggleton 1979; Majerus et al. 1982; Tomlinson et al. 1995). After the experiments, we killed the ladybirds by freezing.

Manipulation

For the first experiment, we chose one male of each sibling pair at random and infected it artificially with

C. hippodamiae larvae, using the methods of Hurst et al. (1995). In the second experiment we chose at random and infected one of each female sibling pair. We manipulated infectious ladybirds, used as a source of *C. hippodamiae* larvae, and each pair of experimental males to expose the underside of the elytra as we did when checking infection status (see above). Then we transferred five to eight larvae to the male (or female in experiment 2) chosen to be infected, using the point of an entomological pin. The uninfected member of each pair was manipulated alongside the infected individual on each occasion. Each male (or female in experiment 2) was then kept separately, moved to a clean petri dish every day to prevent disease, and fed excess *A. pisum*. We checked infection status after 2 days, to ensure infection was successful, and then after 21 days and every 2–3 days afterwards to determine whether the pair were ready for use in the experiment. The pair was deemed ready when the infected individual was heavily infected, that is, adults, eggs and at least 12 of the infective larval stage of *C. hippodamiae* were present. At this stage of infection the virulence effects are expected to be profound. Female *A. bipunctata* kept in the laboratory are typically rendered completely sterile 17 days after infection (Hurst et al. 1995).

Experiment 1: effects on males

The first experiment investigated the effects of infection on male mating success, through both female choice and male vigour, and involved two sets of paired one-on-one tests, where the choice was one of whether or not to mate. A paired test involved placing an uninfected male with an uninfected female in a petri dish and simultaneously placing an infected male with an uninfected female in a separate petri dish and observing the outcomes. We ran the experiment 19 times using different ladybird lines.

In the first test, we compared the interactions of infected and uninfected males with uninfected females that had mated on the previous day. In the second test, conduced 2 h after each male finished mating in the first test, we assessed their interaction with uninfected virgin females. This allowed us to evaluate the effects of infection on male remating ability. We used different categories of females (virgin and previously mated), as mating history might affect the level of female discrimination expressed. Mated females were used first rather than second as these are expected to be more choosy and if they were paired with males potentially made tired by a first test the mating rate might be so reduced as to produce a low sample size.

We did all pairs of tests under constant temperature (25°C), light (equidistant from a lamp) and relative humidity (75%) conditions. In the first test (N=19) we added one female to each of two petri dishes, then added males. Tests ended after 60 min if mating did not occur, or at the end of the mating if it did. Hence, the two pairs of a run of the test were frequently observed for different periods of time. We encouraged mating by transferring the pairs of males and females into new petri dishes every 10 min from 10 min into the test, and by opening the lid of the dish for 30 s every 10 min from 5 min into the test. These actions are thought to maintain concentration gradients of sex pheromones, which males can follow to find females (M. E. N. Majerus, personal communication). The second test with virgin females ensued only if both males mated successfully in the first test (N=16).

For females we recorded (1) the occurrence of rejection behaviour in response to a mating attempt, (2) the duration of rejection behaviour, (3) the number of different types of rejection behaviour and (4) the occurrence of the most effective form of rejection behaviour (pulling up the abdomen). We categorized female rejection behaviour as kicking at the male, lifting the abdomen high above the substrate, rolling, running away, dropping from the lid, shaking and pulling the abdomen up high under the elytra. The first six behaviours can all occur before and after mating begins, defined as when the male inserts his genitalia into the female's genital opening. The seventh form of rejection behaviour, pulling the abdomen up high under the elytra, occurs only before the male begins mating, is the most effective form of rejection behaviour and appears to prevent the male from inserting his genitalia into the female's reproductive tract (de Jong et al. 1993; M. E. N. Majerus, personal observation).

For males we recorded (1) the occurrence of a mating attempt, that is, trying to mount the female, (2) the time to the first mating attempt, (3) the duration of mating and (4) the number of spermatophores the male transferred during the mating. Ransford (1997) showed that male *A. bipunctata* can produce multiple spermatophores during a single mating. There are cycles of stereotypical male behaviour corresponding to each transfer. Each cycle comprises two phases, a 'twisting phase' and a 'rocking phase' with distinct male movements. The number of spermatophores transferred during a copulation can be assessed by observing this behaviour and counting the number of spermatophores expelled from the female's tract (Ransford 1997). Both the female and the male may influence duration of mating and number of spermatophores transferred. However, it is most likely that they represent the level of male investment in the mating, and that females have little control over the duration of matings once intromission has been achieved (Ransford 1997).

Experiment 2: effects on females

The second experiment investigated the effect of infection on female attractiveness and willingness to mate. We compared the outcomes of pairings of uninfected males with infected and uninfected females.

The protocol was essentially the same as that of experiment 1. We used a full-sib, paired design involving one-on-one tests. In this case, however, one of the females used in each replicate was infected, whilst both males were uninfected. Additionally, we did only one test. The experiment was repeated 22 times. The males were mated on the day before the tests as this was expected to increase choosiness. Virgin male insects are notoriously unselective in their choice of mates (Thornhill & Alcock 1983). In many insect species

Table 1. Infection and mating status of males and females collected from the field in 1997 and 1999

	Males		Females	
	Uninfected	Infected	Uninfected	Infected
1997				
N	16	45	15	48
% Mating	37.5	40	46.7	35.4
95% confidence limits				
Lower	18	27.5	24.5	24
Upper	61	53	70	48
χ^2_1 corrected for continuity	<0.001		0.23	
P	>0.999		0.63	
1999				
N	221	72	224	79
% Mating	11.3	12.5	7.6	7.6
95% confidence limits				
Lower	8	6.5	5	3.5
Upper	15.5	20.5	11	14.5
χ^2_1 corrected for continuity	0.004		<0.001	
P	0.95		>0.999	

including ladybirds, males deprived of females are liable to attempt matings with unsuitable partners such as other males or dead females (K. M. Webberley, personal observation). We recorded male and female behaviour as described in experiment 1.

Statistical analysis

We used χ^2 and Fisher's exact tests (for smaller samples) to investigate the effects of the infection status of the male (experiment 1) and the female (experiment 2) on the frequency of the different male and female behaviours and the outcomes of pairings. We compared the duration of rejection behaviours, time to first mating attempt and duration of matings for uninfected and infected groups using Wilcoxon signed-ranks tests. In the case of rejection behaviour, data were used only where both males of a paired test attempted to mate. Two-tailed statistical tests were used throughout. Two packages were used for the analyses: Fish6 for the Fisher's exact tests and StatView for χ^2 tests and Wilcoxon tests where $N>15$.

Our four measures of female behaviour corresponding to female willingness to mate were occurrence of rejection behaviour, duration of rejection behaviour, occurrence of abdomen tucking and the total number of different forms of rejection behaviour. These may be regarded as multiple tests of the same hypothesis, namely infection status of either the male (experiment 1) or female (experiment 2) has no effect on female propensity to mate. To avoid spurious rejection of the null hypothesis purely because of the large number of statistical tests, we corrected the *P* value of each test using the Bonferroni procedure (Weir 1990). Similarly, in each experiment (or test in the case of experiment 1), we had two measures of male propensity to mate and three measures of overall success. The *P* values were corrected accordingly for tests of each hypothesis.

RESULTS

Field Mating Rates

There was no significant association between infection status and mating status in the field for either males or females, in either 1997 or 1999 (Table 1). The proportions of infected and uninfected individuals found in copula were remarkably similar in both sexes in the larger sample from 1999 (Table 1). Results were also nonsignificant when the data from the five weekly collections of 1999 were analysed separately.

Laboratory Experiments

Experiment 1: Effects on males

Female behaviour. There was no strong evidence for an effect of male infection status on female willingness to mate, that is, no evidence of mate choice on the basis of infection status. The occurrence of any form of mate rejection behaviour by previously mated (test 1) or virgin females (test 2) was independent of male infection status (Table 2). Similarly, there was no significant difference in the number of different forms of rejection behaviour of females in response to mating attempts by infected males and uninfected males (Table 2).

There was no significant difference between the duration of rejection behaviour of either previously mated or virgin females in response to mating attempts by males of different infection status (Table 2). In these tests the duration of rejection behaviour appeared to be heavily influenced by females rather than males, and thus is a good indication of female willingness to mate. The males abandoned attempts as a result of female rejection behaviour in only two cases with mated females (test 1) and four cases with virgin females (test 2).

Previously mated females (test 2) were more likely to show the strongest form of rejection behaviour (tucking

the abdomen high up under the elytra) in response to mating attempts by infected rather than uninfected males (Table 2). However, this difference was only significant at the 5% level without Bonferroni correction and thus is best considered a consequence of the large number of statistical tests performed and not as an indicator of a mating preference. In addition, male infection status had no effect on the rate at which virgin females performed this behaviour (Table 2).

Male behaviour. No significant effect of infection on male vigour, willingness to mate or remating ability was discernible from the tests. Whether or not a male attempted to mate was independent of the infection status of the male in both tests (Table 3). In addition, there was no significant difference in time to first mating attempt in either test (Table 3).

Overall mating success. The crucial test of the effect of infection on host mating behaviour is the final outcome of pairings (either a successful mating or no mating). This was independent of the infection status of the male both with previously mated females (test 1) and with mated females (test 2; Table 4). The success rates of infected and uninfected males were identical in test 1.

The sample sizes in the tests are moderate, owing to the use of a carefully controlled experiment with full-sib design, and an obvious question is how powerful could they be in identifying an effect. However, retrospective power analysis for an observed effect size is generally not considered to be very useful (Thomas & Krebs 1997). Certainly, cursory examination of the power of these tests would be misleading. The power of Fisher's exact tests decreases with both decreasing sample size and increasing similarity of results for the two groups. Indeed, when test outcome is exactly as predicted by the null hypothesis, as in test 1, the power of the test (probability of rejecting a false null hypothesis) is very low and would not be increased substantially by increasing the sample size.

An alternative analysis of the experiment's sensitivity is to calculate the minimum effect that would produce a significant deviation from the null hypothesis given the sample sizes used. In the case of test 1 and a fixed success rate of 89.5% for uninfected males, a success rate of 53% for infected males is the maximum success rate that would have produced a significant result at the 5% level (with Bonferroni correction). This requires a large deleterious effect, but one would expect that if the parasite decreases male libido, or if there is female choice for uninfected partners, a large effect on male mating success would be evident.

The 'power' of the experiment to identify any effects of infection on male mating success is increased by the use of two tests rather than just one. In the second test with virgin females the sample size could have allowed us to identify a 38% success rate for infected males compared to 75% for uninfected males. The second test could also have allowed us to identify a positive effect of infection on male mating success rate.

There was no significant difference in the duration of mating for infected and uninfected matings in the two tests (Table 4). Nor was there any significant difference in number of mating cycles (corresponding to number of spermatophores transferred; Table 4).

Experiment 2: Effects on females

Male behaviour. There was no evidence that female infection status affected their attractiveness to males. The majority of males attempted to mate with the females they were paired with. There was no significant difference between the number of males paired with uninfected females that attempted to mate and those paired with infected females that attempted to mate (Table 5). There was a nonsignificant trend for males paired with uninfected females to attempt mating sooner than males paired with infected females (Table 5).

Female behaviour. It appears that infected females were no more or less willing to mate than uninfected females. Both infected and uninfected females commonly showed at least one form of rejection behaviour in response to male mating attempts, and there was no association between the frequency of rejection behaviour and the infection status of the females (Table 6). Similarly, the durations of rejection behaviour of infected and uninfected females were not significantly different (Table 6). Although a greater proportion of the infected females tucked their abdomens up under their elytra in response to a mating attempt, the difference was not significant at the 5% level (Table 6). The number of different types of rejection behaviour of infected females and uninfected females in response to mating attempts was not significantly different (Table 6).

Final outcome. Overall, the final outcome of the tests, mating or lack of mating, was independent of female infection status (Table 7). Given the sample size and assuming a fixed success rate for uninfected females of 77.3%, the minimum effects of infection that could have produced significant results at the 5% level with Bonferroni correction are a decrease in the success rate to 41% and an increase to 100%. The duration of matings also did not differ with female infection status (Table 7). The number of cycles of male mating behaviour, corresponding to the number of spermatophores transferred, did not differ for males paired with uninfected and infected females (Table 7). This indicates that males invested equally in matings with infected and uninfected females.

DISCUSSION

No Evidence of Parasite-mediated Effects on Mating Behaviour

Parasites are known to affect host mate choice and mating patterns (see Kavaliers et al. 2000 for a review; but also Kraaijeveld et al. 1997; Rolff et al. 2000). However, there has been limited investigation of the effects of

Table 2. The behaviour of uninfected females when presented with uninfected or infected males in one-on-one choice tests in experiment 1

Female behaviour in response to male attempt	Female mating experience prior to test*	% Females showing behaviour or mean response level±SE (*N*†)		Statistical test	Statistical value‡	*P* two tailed	*P* with Bonferroni correction
		Infected males	Uninfected males				
Acceptance (no rejection behaviour)	Mated	11.1 (18)	22.2 (18)	Fisher's exact	—	0.66	0.89
	Virgin	20.0 (15)	6.7 (15)	Fisher's exact	—	0.35	0.82
Abdomen raised under elytra	Mated	61.1 (18)	38.9 (18)	χ^2	4.11	0.04	0.16
	Virgin	20.0 (15)	20.0 (15)	χ^2	<0.001	>0.99	1.00
Duration of rejection (s)§	Mated	465±258 (18)	492±220 (18)	Wilcoxon	−0.065	0.95	1.00
	Virgin	131±55 (15)	131±57 (15)	Wilcoxon (*N*=14; scores equal for 1 pair)	62	0.58	0.97
No. of different types of rejection behaviour§	Mated	2.6±0.4 (18)	2.1±0.5 (18)	Wilcoxon (*N*=14; scores equal for 4 pairs)	58	0.76	0.99
	Virgin	2.1±0.4 (15)	2.0±0.3 (15)	Wilcoxon (*N*=12; scores equal for 3 pairs)	44	0.73	0.99

*Males were used in up to two tests. They were first presented with previously mated uninfected females (test 1). If both paired males mated then a second test with uninfected virgin females took place (test 2).
†*N*=total number of runs in which males attempted mating.
‡χ^2_1 corrected for continuity or Wilcoxon T^+ when $N \leqslant 15$ or Wilcoxon *Z* corrected for ties when $N > 15$.
§Data from tests where the females did not reject at all in response to male mating attempts are included in the calculations of the mean duration of rejection behaviour and the mean number of types of rejection behaviour.

Table 3. The behaviour of uninfected and infected males when presented with uninfected females in one-on-one choice tests in experiment 1

Male behaviour	Female mating experience prior to test*	% Males showing behaviour or mean response level± SE (*N*†)		Statistical test	Statistical value‡	*P* two tailed	*P* with Bonferroni correction
		Infected males	Uninfected males				
Mating attempt	Mated	94.7 (19)	94.7 (19)	Fisher's exact	—	1.00	1.00
	Virgin	93.8 (16)	93.8 (16)	Fisher's exact	—	1.00	1.00
Mean time to attempt (s)§	Mated	647±222 (19)	666±203 (19)	Wilcoxon (*N*=18; scores equal for one pair)	−0.109	0.91	0.99
	Virgin	629±249 (16)	503±222 (16)	Wilcoxon (*N*=15; scores equal for one pair)	50	1.00	1.00

*Males were used in up to two tests. They were first presented with previously mated uninfected females (test 1). If both paired males mated then a second test with uninfected virgin females took place (test 2).
†*N*=total number of males.
‡Wilcoxon T^+ when $N \leqslant 15$ or Wilcoxon *Z* corrected for ties when $N > 15$.
§Data from runs in which the male failed to attempt mating are included in the calculation of mean time to first mating attempt. In these cases the interval was underestimated as 1 h.

Table 4. The final outcome of pairings in experiment 1, in which either uninfected males or infected males were presented to uninfected females

Outcome of pairings	Female mating experience prior to test*	% Runs showing outcome or mean outcome±SE (*N*†)		Statistical test	Statistical value‡	*P* two tailed	*P* with Bonferroni correction
		Infected males	Uninfected males				
Successful mating	Mated	89.5 (19)	89.5 (19)	Fisher's exact	—	1.00	1.00
	Virgin	81.3 (16)	75.0 (16)	Fisher's exact	—	0.70	0.97
Mean duration of mating (min)§	Mated	149±21 (19)	168±23 (19)	Wilcoxon (*N*=17; scores equal for 2 pairs)	−0.876	0.38	0.76
	Virgin	63.6±14 (16)	77.2±15 (16)	Wilcoxon (*N*=14; scores equal for 2 pairs)	65	0.46	0.85
Mean no. of insemination cycles§	Mated	1.63±0.2 (19)	1.79±0.2 (19)	Wilcoxon (*N*=10; scores equal for 9 pairs)	34	0.56	0.91
	Virgin	0.94±0.2 (16)	1.00±0.2 (16)	Wilcoxon (*N*=7; scores equal for 9 pairs)	15.5	>0.81	0.99

*Males were used in up to two tests. They were first presented with previously mated uninfected females (test 1). If both paired males mated then a second test with uninfected virgin females took place (test 2).
†*N*=total number of males.
‡Wilcoxon T^+ when $N \leqslant 15$ or Wilcoxon *Z* corrected for ties when $N > 15$.
§Data from those runs where mating did not occur are included in the calculations of mean mating duration and mean number of insemination cycles.

Table 5. Male behaviour when presented with uninfected or infected females in one-on-one choice tests in experiment 2

Male behaviour	% Males showing behaviour or mean interval±SE (*N**)		Statistical test	Statistical value†	*P* two tailed	*P* with Bonferroni correction
	Infected females	Uninfected females				
Mating attempt	77.3 (22)	90.9 (22)	Fisher's exact	—	0.25	0.44
Mean time to attempt (s)‡	1283±298 (22)	805±234 (22)	Wilcoxon (*N*=21; scores equal for one pair)	1.755	0.08	0.15

**N*=total number of males.
†Wilcoxon *Z* corrected for ties.
‡Data from runs in which the male failed to attempt mating are included in the calculation of mean time to first mating attempt. In these cases the interval was underestimated as 1 h.

Table 6. The behaviour of uninfected and infected females when presented to uninfected males in one-on-one choice tests in experiment 2

Female behaviour in response to male attempt	% Females showing behaviour or mean response level±SE (*N**) Infected females	Uninfected females	Statistical test	Statistical value†	*P* two tailed	*P* with Bonferroni correction
Acceptance (no rejection behaviour)	11.8 (17)	25.0 (20)	Fisher's exact	—	0.67	0.99
Abdomen raised under elytra	70.1 (17)	50.0 (20)	Fisher's exact	—	0.32	0.78
Mean duration of rejection (s)‡	220±123(17)	87.5±21(20)	Wilcoxon signed-ranks (*N*=15; both males attempted in 16 runs; scores equal for 1 pair)	60	1.00	1.00
Mean no. of different types of rejection behaviour‡	2.18±0.4 (17)	2.20±0.4 (20)	Wilcoxon signed-ranks (*N*=9; both males attempted in 16 runs; scores equal for 7 pairs)	18	1.00	1.00

**N*=total number of runs in which males attempted mating.
†Wilcoxon T^+.
‡Data from tests where the females did not reject at all in response to male mating attempts are included in the calculations of the mean duration of rejection behaviour and the mean number of types of rejection behaviour.

Table 7. The overall outcome of one-on-one matings tests in experiment 2, in which uninfected males were presented to either infected or uninfected females

Outcome of pairings	% Pairings showing outcome or mean outcome± SE (*N**) Infected females	Uninfected females	Statistical test	Statistical value†	*P* two tailed	*P* with Bonferroni correction
Successful mating	68.2 (22)	77.3 (22)	Fisher's exact	—	0.52	0.89
Mean duration of mating (min)‡	58±12 (22)	51±7 (22)	Wilcoxon signed-ranks (*N*=18; scores equal for 4 pairs)	−0.022	0.98	0.99
Mean no. of insemination cycles‡	0.9±0.2 (22)	0.8±0.1 (22)	Wilcoxon signed-ranks (*N*=10; scores equal for 12 pairs)	30	0.85	0.99

**N*=total number of males.
†Wilcoxon T^+ when $N \leq 15$ or Wilcoxon *Z* corrected for ties when $N > 15$.
‡Data from those runs where mating did not occur are included in the calculations of mean mating duration and mean number of insemination cycles.

purely sexually transmitted parasites on host mating behaviour. Changes are expected as a result of the direct negative effects of the parasite and consequent adaptations in both the host and the parasite. Abbot & Dill (2001) found no evidence for mate choice in *L. clivicollis* on the basis of STD infection status, but did find that infected males invested more in reproduction via increased aggression and competitive ability. We looked for effects of a sexually transmitted parasite in a host with a different mating system, in which other host adaptations may be more likely. In contrast to *L. clivicollis*, intrasexual selection in *A. bipunctata* appears weak and there is more opportunity for intersexual selection. Although we carried out a large multifaceted study involving both field work and controlled laboratory experiments, we found no evidence for any effects of *C. hippodamiae* infection on *A. bipunctata* male or female mate choice or willingness to mate. We therefore conclude that effects are either small or absent.

Perhaps most surprising was the lack of a preference for uninfected partners in either sex. Past exploration of the potential role of parasites in the evolution of mating preferences has provided strong evidence for the importance of parasites in mate choice. Theoretical studies have suggested that direct selection, for example for disease avoidance, may be particularly important in the evolution of mating preferences (Price et al. 1993; Kirkpatrick & Barton 1997). Parasite transmission avoidance is perhaps most likely to evolve in the presence of an STD. Here choice of an uninfected mate has clear direct benefits. Several factors indicated that mating preferences would be expected within the specific system used. First, during the early part of the season, when infection has the greatest consequences, between 20 and 40% of adults of both sexes are infected (K. M. Webberley, unpublished data). Thus, uninfected individuals are likely to come into contact with infected partners. Second, mating with an infectious partner is extremely likely to result in parasite transmission. Under laboratory conditions transmission efficiency is 96.4% (Hurst et al. 1995). Third, infection carries a high cost: sterilization in female hosts. The incubation period of this effect (17 days) is short relative to the reproductive life span of female *A. bipunctata*, which may be a couple of months. The mite develops at the same fast rate on male hosts and, at least during the stressful conditions of overwintering, infected males have reduced survival. Fourth, both sexes are able to exert some control over matings. Males initiate mating by climbing on females. However, effective female rejection behaviour is observed in the field and past laboratory studies have suggested female choice (Majerus et al. 1982; Majerus 1994a). Our surprising findings lead us to ask two questions. First, why is there a lack of mate choice? Second, are there alternative host behavioural adaptations to STD presence in this system?

Possible Explanations for the Lack of Mate Choice

Three types of 'constraints' might have prevented the evolution of mate choice. The most obvious are sensory constraints. Males and females may not be able to assess the infection status of prospective partners through direct indicators. Although we could, in some cases, see the mite colonies through the elytra, it is possible that *A. bipunctata* cannot do so. Foraging studies have shown that coccinellids have poor visual acuity (Stubbs 1980). The nature of any chemical cues or physical changes associated with *C. hippodamiae* infection are unknown. Infection in vertebrates may produce a stronger cue, or one more directly linked with sexual attractiveness, than infection in invertebrates, as the immune system and sexual activity are frequently linked in vertebrates through testosterone activity. This may explain why preferences are observed in laboratory mice but not here. Certainly, infection has large effects in mice; it alters immune, neurochemical and endocrine function, for example, reducing testosterone production (Kavaliers et al. 2000).

A second type of constraint is counteradaptation in the parasite. If there had been host mate choice on the basis of infection status in the evolutionary past of *C. hippodamiae* it would have been under strong selection to be cryptic. Thus, the lack of mate choice may indicate parasite adaptation. In addition, the mite did not reduce its host's mating activity, by an effect on attractiveness or willingness to mate, which also appears adaptive. However, we found no evidence of the ultimate parasite adaptation to sexual transmission: *C. hippodamiae* did not increase the libido of either male or female hosts or host attractiveness. This may be because as an ectoparasite rather than an endoparasite, its influence on the host is constrained. Alternatively, male libido at least may already be at maximal levels in this species. A high proportion of males attempted mating in both experiment 1 (94.7% in test 1) and experiment 2 (84%).

A third possible explanation for the lack of mate choice is that the high cost of making a choice constrains its evolution. Female rejection behaviour is often prolonged and probably energetically costly. Thus, strong selection pressures or a reduction in costs may be needed for it to evolve. It may be least costly and most effective when aimed at weak males, which is perhaps why small (Tomlinson et al. 1995) or ill-fed males (Majerus 1994a) are at a mating disadvantage in this species. One would expect that *C. hippodamiae* infection would also weaken males. However, the fact that males are not rejected more readily indicates that this is not the case, perhaps as a result of parasite adaptation. Thus, although female *A. bipunctata* are likely to be under strong selection to avoid a sterilizing infection, evolution of a female mating preference on the basis of male infection status may be selected against by the high costs of rejection.

Evolution of a mating preference for uninfected partners may also be constrained in males by the costs of choosing. Male insects are typically under strong selection to mate frequently with many females (Bateman 1948). It is likely that the net benefits of male choice are reduced by the time costs associated with this strategy.

Alternative Host Adaptations to STD Presence

Although we found no evidence of mate choice on the basis of infection status within our study system, one or more other adaptations to STD presence might have evolved. Alternative adaptations include immunological and chemical resistance and also behavioural changes.

The obvious alternative behavioural adaptations are reductions in mating duration (Hamilton 1900; Sheldon 1993) and rate. The long matings we observed (Tables 4, 7) and the high transmission efficiency of *C. hippodamiae* indicate that shorter mating has not evolved. This may be because mating duration is a key factor in determining sperm competition success in *A. bipunctata* (Ransford 1997). Similarly, the likelihood of reduced promiscuity evolving depends heavily on the other costs and benefits of mating (Thrall et al. 1997) and sexual conflict is likely.

Other possible adaptations to *C. hippodamiae* involve changes in life history. Presence of a sterilizing or lethal parasite in a population is expected to select for an increase in early reproductive effort, to compensate for the future loss of reproductive success (Minchella & Loverde 1981; Minchella 1985; Shykoff & Kaltz 1997). This is possible in female *A. bipunctata* as they store large amounts of sperm (Majerus 1994b) and hence could increase reproduction without increasing parasite exposure. Alternatively, infection might trigger increased reproductive effort by individual females, much as infection in male milkweed leaf beetles leads to increased aggression and competitive ability. Infection did not cause any obvious increase in reproduction allocation in male ladybirds. This is probably due to differences in the mating system. Male mating rate is probably already at maximal levels in *A. bipunctata* (see above) and there is little or no direct male–male aggression.

There has been growing speculation about the potential evolutionary effects of STDs on host mating systems and life history strategies (Sheldon 1993; Lockhart et al. 1996). To date, the speculation has exceeded empirical data. It is notable that existing studies, including this one, suggest that mate choice as an adaptation to STD presence may be less likely in invertebrate STD systems than vertebrate ones. The question now must be what features constrain the evolution of mate choice. In addition, combined with work on another beetle–mite system, our work indicates that adaptations to STD presence may be highly dependent on the particular mating system of the host. Clearly, work is needed on this and other STD systems before we can fully understand the many evolutionary implications of sexual transmission for parasites and their animal hosts.

Acknowledgments

K.M.W. was funded through a BBSRC studentship and subsequently through NERC grant GR3/11818. G.D.D.H. is a BBSRC David Phillips Research Fellow. Part of the work was carried out in a laboratory funded by the Wolfson Foundation. We are also grateful to Dr Cahir O'Kane for the use of his constant temperature room and to Dr Andrew Pomiankowski and the anonymous referees for comments on the manuscript.

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