

Sexually transmitted parasites and sexual selection in the milkweed leaf beetle, *Labidomera clivicollis*

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Milkweed leaf beetles (MLBs; *Labidomera clivicollis* Chrysomelidae) are parasitized by a subelytral mite, *Chrysomelobia labidomerae* (Tarsonemina: Podapolipidae). We show that *C. labidomerae* is transmitted between MLBs when they copulate, and can reduce the survival of nutritionally stressed beetles. We investigated the effect of this sexually transmitted parasite on mate choice and male-male competition in MLBs, and the consequences of variation in these behaviours for mite transmission. We found no evidence of parasite avoidance by MLBs, and evidence for high rates of parasitism in the MLB populations we surveyed. In the absence of females, parasitized males contacted unparasitized males more often and for longer than controls, and they tended to displace rival males from females more often than did unparasitized males, a result consistent with the interpretation that parasitized beetles compensate for loss of fitness by increasing reproductive effort. These changes can also benefit mites, because longer and possibly more contacts between beetles provide more opportunities for transmission, but there is no evidence that these changes in male behaviour result from parasite manipulation.

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Because they require close proximity between hosts for transmission, infectious diseases can play decisive roles in the sexual and social behaviour of their hosts. In recent years both conceptual and empirical research have grown to incorporate the fact that infectious parasites, especially sexually transmitted parasites, may be common, if cryptic, factors shaping the mating biology of many organisms (Loehle 1995, Lockhart et al. 1996). A number of studies have emphasized the various effects of infectious diseases on both host secondary sexual characteristics (e.g., Knell 1999) and mate choice (e.g., Able 1996, Loehle 1997, Kiesecker et al. 1999), as well as the direct effects of parasitism on host competitive ability, life history trade-offs, and reproductive effort (e.g., Forbes 1991, 1993, Polak and Markow 1995, Thomas et al. 1999). An aspect of the interaction that has received comparatively less atten-

tion is how the effects of disease (or risk of disease) on host interactions influence the population biology of the parasites themselves (Møller 1996, Knell 1999). For example, from the perspective of a contagious parasite, any host behaviours which alter the frequency of contacts between parasitized and unparasitized hosts can reduce the rate of infectious spread through the host population, and potentially depress the growth rate of the parasite population. For harmful parasites, such host behaviours may occur commonly: unparasitized hosts may avoid parasitized mates, for example, or parasitized hosts may exhibit less competitive ability than unparasitized counterparts, reducing the frequency of direct interactions with other hosts (Loehle 1995, Zuk and McKean 1996).

Infectious parasites and their hosts thus can provide unique opportunities to explore reciprocal interactions

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between host behaviour and parasite population biology (Richner 1998, Clayton et al. 1999). Little empirical evidence exists, however, that addresses the extent to which an infectious disease influences host sexual behaviour, in conjunction with the extent to which those host sexual behaviours affect parasite transmission. Here we consider the joint effects of mate choice and male-male competition on the transmission biology of a sexually transmitted parasite and simultaneously ask how the parasites affect these same behaviours.

We investigated mate choice and male-male competition for females in the milkweed leaf beetle (MLB) *Labidomera clivicollis*, parasitized by the mite *Chrysomelobia labidomerae* Eickwort (Acari: Tarsonemina: Podapolipidae). MLBs occur on milkweed (*Asclepias* spp. and *Cynanchum* spp.) throughout the eastern U.S. and Canada. Like many chrysomelid beetles, MLBs are characterized by multiple matings, prolonged pairing between males and females, and mixed paternity broods (Dickinson 1986, 1988, 1992, 1997). MLBs typically experience only one long season (May–September) favourable to reproduction, in which two or three overlapping generations are produced. Males competitively search for and monopolize females ('scramble competition polygyny'; Dickinson 1992), often foregoing feeding to do so, and assure paternity by guarding females until oviposition, and at times inseminating females repeatedly. Once a male encounters a female, there is little or no courtship before mating. Male MLBs occasionally fight one another, normally when a solitary male attempts to displace a copulating male, but fights sometimes occur in the absence of females, possibly to evict rivals from plants.

The family Podapolipidae consists of highly specialized insect parasites that feed on their hosts by piercing the cuticle with their styliform chelicerae (Baker and Eickwort 1975, Hurst et al. 1995). *C. labidomerae* is a subelytral beetle parasite first described on New York state MLBs (Eickwort 1975). *C. labidomerae* has since been described on *Labidomera* spp. throughout North America, and morphologically-indistinguishable species of mites have been described on *Leptinotarsa* spp. over the same range.

Female *C. labidomerae* lay eggs on the wings of the host, laying on average 1–2 eggs per day (Baker and Eickwort 1975, Drummand et al. 1988). Larvae mature at the base of the wings or in the thoracic crevice, where they feed (Regenfuss 1972). The engorged nymphs then migrate to the ventral surface of the elytra, and develop into adults. Adult females overwinter under the elytra of diapausing adult beetles, apparently synchronizing their diapause to match that of their hosts (Baker and Eickwort 1975, Drummand et al. 1988). With no free-living stage, *C. labidomerae* cannot survive off their host for more than a few minutes to a few hours, and mite numbers can grow quite large (Baker and Eickwort 1975, Drummand et al. 1989).

Baker and Eickwort (1975) reported an average of 11 mites per beetle in the field, with laboratory densities ranging as high as 194 mites per beetle. Previous studies have suggested that transmission occurs only by direct contact between adult beetles: before transmission, adult female mites typically move from beneath the elytra to the abdomen of MLBs and migrate to new hosts either during copulation or occasionally during interactions between host individuals of the same sex (fighting, for example; Baker and Eickwort 1975, Drummand et al. 1988). Results concerning how the parasite affects survival have been more equivocal (Eickwort and Eickwort 1986, Drummand et al. 1989).

We investigated sexual transmission by *C. labidomerae* and measured MLB survival under parasitism experimentally. We then asked if and how parasitism influences the sexual behaviour of beetles by measuring the effect of mites on mate choice and male-male competition. In the mate choice experiments, we anticipated that unparasitized mates would avoid infectious partners to minimize the likelihood of receiving parasites (Able 1996, Loehle 1997). In the experiments on male-male competition, the most straight-forward expectation was that, because mites feed on male haemolymph, parasitized males would be less competitive than their unparasitized counterparts (Siva-Jothy and Plaistow 1999, Thomas et al. 1999). However, the range of possible direct effects of parasites on host behaviour extends beyond the pathological, and includes functional changes that benefit hosts, parasites, or both (Moore and Gotelli 1990, Poulin et al. 1994, Lafferty 1999). In particular, we bore in mind the possibility that male MLBs may express some degree of functional plasticity in competitive behaviour, as a means to compensate for the loss of fitness due to parasitism (Forbes 1993). In order to identify such plasticity (should it occur), we paid particular attention to those effects that altered the outcome of competition, because these are relevant to both parasitized male fitness and to mite transmission opportunities.

Finally, after measuring the effect of the parasite on MLBs, we asked if variation in parasite transmission is potentially correlated with the observed variation in host sexual behaviour, and if mite prevalence in a wild population is consistent with the observed host behaviours.

Methods

Laboratory methods

Approximately 250 milkweed leaf beetles were collected from the Queen's University Biological Station at Elgin, Ontario, Canada (44°34' N, 76°19' W), and from the Cornell Biological Field Station near Bridgeport, New York, USA (43°09' N, 75°58' W). *Asclepias* and

beetles were locally abundant at both field sites. We bred beetles from both populations in the laboratory, producing a stock of approximately 250 beetles, which were maintained separately in Petri dishes in an environmental chamber at 25°C with a 16:8 L:D cycle and at high humidity – conditions approximately like those in southern Ontario and northern New York in mid-summer. We cultured mites on separate beetles until needed for experiments. All parasitized beetles used in behavioural experiments were parasitized with 20 ± 2 adult female mites at least one month before experiments began. This number of mites was judged to be adequate to insure that our experimental beetles had at least the average number of mites measured in field surveys, assuming not all would survive or reproduce after being transferred to new hosts (Baker and Eickwort 1975). We controlled for this handling of parasitized beetles by tapping the elytra of all the unparasitized beetles with a clean '000' pin.

Parasite transmission and host survival

To determine if mites are sexually transmitted, we paired parasitized males with unparasitized females in such a way that the only contact between the two occurred via copulation, and quantified both the infection rates of females and the relationship between contact duration and mite transmission. Three groups of parasitized males of equivalent size and known parasite number were allowed to mate with females for durations of 60 s ($n = 10$), 240 s ($n = 9$), or 900 s ($n = 9$). We scored females for mites, and then counted the number of mites remaining on each male.

In the survival experiments, we reasoned that a potentially important source of variation may lie in the fact that the adverse effects caused by parasites can vary with host condition (Forbes and Baker 1991, de Lope et al. 1993, Yan and Stevens 1995, Polak 1996, Jokela et al. 1999). We therefore investigated the effect of the mites on MLB longevity when the beetles were nutritionally stressed. We did not quantify fecundity or grooming in these experiments (Hart 1994, Leonard et al. 1999). Our observations suggested that MLBs have little ability or inclination to remove mites: we never observed mites dislodged from newly parasitized beetles.

We selected 32 (16 male/16 female) two-week-old adult virgin MLBs of similar size from the laboratory stock, and randomly chose approximately one half of the individuals of both sexes to be experimentally infested with 20 ± 2 adult female mites. The beetles were fed equivalent pieces of milkweed (approx. 4 cm²) every four to five days and observed daily for approximately six months. Survival distributions of the two groups were compared by the LIFETEST Procedure (SAS Institute, Inc. 1985). Reported chi-square values are for Wilcoxon statistics (SAS Institute, Inc. 1985).

To measure the effect of mites on starved beetles, we selected 36 two-month-old male MLBs of similar size and ceased feeding them for 14 d; 18 randomly chosen from this group were infested with 20 ± 2 adult female mites.

Mate choice under risk of parasitism

We tested for parasite avoidance in MLBs using both laboratory and field populations. In the laboratory, we conducted dichotomous preference tests for both males and females on an erect wooden Y-maze, 35 cm high by 46 cm wide, which was enclosed in a small test arena at 25°C and illuminated by fluorescent lamps. We made no specific assumptions about means of parasite detection by MLBs. Relative to bacteria and viruses, *C. labidomerae* are large and not especially cryptic parasites. Parasitized beetles have lesions and open wounds at feeding sites beneath the elytra, as well as decaying egg sacs, molts, and other detritus that could serve as reliable chemosensory signals of the presence of mites (Baker and Eickwort 1975, Eickwort 1975).

For male mate choice experiments, 12 unparasitized, virgin females of similar weight were chosen from the laboratory stock and six of these were randomly parasitized with 20 ± 2 adult female mites. Thirty-six unparasitized virgin male MLBs of the same age were each randomly presented with one of the 36 possible combinations of the six unparasitized and six parasitized females, each member of a female pair being tethered to one end of the Y-maze. Choice was defined by the relative amount of time a male spent on either arm of the maze, using 'commitment' lines drawn approximately two body lengths from each female. All trials were videotaped, and we recorded the frequency of contacts by each male with the two females, and whether the contact resulted in the male successfully mounting the female. Trials ended when males dropped off the apparatus or mounted a female, or 15 min had elapsed.

Female mate choice experiments differed only slightly. Reasoning that expression of choice may depend on the female's estimate of the availability of unparasitized mates in the population, we paired 36 unparasitized females individually with unparasitized virgin males for two days before the experiment. Each female was then maintained on a milkweed plant for 24 h to ensure she was well fed before the experiment. We randomly presented the 36 females to 36 combinations of 12 previously prepared males, half of which were experimentally parasitized with 20 ± 2 adult female mites. All trials were videotaped and subsequently scored for the amount of time spent and the frequency of contacts (as defined above) by females with either male. Each trial ended when females either dropped off the apparatus, or were mounted by a male, or after 15 min.

In order to test for mating with respect to parasites in a wild population, we conducted a field survey of the distribution of parasitized and unparasitized individuals with respect to sexual activity. We reasoned that if avoidance of parasitized mates were pronounced, then pairs in copula would exhibit lower rates of parasitism relative to the background frequency in the local population (Thomas et al. 1999). We compared the incidence of mites on copulating pairs with the incidence of mites on singletons of each sex. Two populations of MLBs on *A. incarnata* were sampled at the Queen's University Biological Station (QUBS). For each pair collected, two singletons were collected from the nearby vegetation whenever possible. All beetles were transported separately in sealed vials, and scored for the presence or absence of mites at Simon Fraser University.

Male-male competition and parasitism

We measured how parasitism affects male aggression toward other males (in the absence of females) by scoring both the frequency and duration of contacts between parasitized and unparasitized male beetles. One hundred and six virgin males were chosen from the laboratory stock and 30 were randomly chosen from this group and experimentally parasitized by 20 ± 2 adult female mites. The beetles were divided into four groups: (1) 30 parasitized 'test' males paired with (2) 30 unparasitized males, and (3) 23 unparasitized 'test' males paired with (4) 23 unparasitized males. We refer to the 30 pairs of parasitized and unparasitized males as the 'parasitized group', and the 23 pairs of unparasitized males as the 'control group'. We refer to the two males in each trial as the 'test' male (parasitized or unparasitized), whose behaviour we measured, or the 'rival' male (unparasitized). In the control group, one member of each pair was randomly chosen to be the test male.

Trials were conducted in 5-cm-diameter plastic Petri dishes, and lasted for two hours. We videotaped each trial, and blindly scored: (1) the frequency of contacts initiated by the test male with the rival male, and (2) the duration of such contacts. Because beetles are most active at night, all trials were started at night in a darkened laboratory and filmed under infrared light. To facilitate analysis, one beetle in each dish had been randomly chosen and marked with correction fluid on an elytron, at least six hours before the beginning of the trial. Liquid correction fluid did not appear to have any effect on beetle behaviour, and was easily removed from the elytra after the trial. We counted the number of mites on all beetles at the end of each trial.

To determine the effect of *C. labidomerae* on male behaviour towards copulating pairs, we measured the frequency of contacts by parasitized and unparasitized males with such pairs, and the success with which both

types of male displaced copulating males from females. Thirty-six test males of the same age and size were randomly chosen from the laboratory stock, and half were experimentally parasitized with 20 ± 2 adult female mites. We placed 36 unparasitized virgin males with 36 unparasitized virgin females in Petri dishes and allowed them to copulate. All trials were started at night in a darkened laboratory and filmed under infrared light. Approximately one hour after the beetles were paired, we introduced a test male (parasitized or unparasitized) to each petri dish. Trials were videotaped for six hours and we later blindly scored each trial for: (1) the number of contacts by the test male with the rival male; (2) the duration of each contact by the test male with the rival male; (3) whether or not the rival male was displaced from the female in a trial; and (after successful takeovers) (4) contact rates and durations with test males by the rival males in each trial. We again counted the number of mites on all beetles at the end of each trial.

Results

Parasite transmission and host survival

Mean mite numbers on males before copulation were 42.5 ± 11.8 SE, 49.8 ± 12.0 SE, and 46.2 ± 16.4 SE for the 60, 240, and 900 s copulation groups, respectively. For all durations combined, 21 of 28 females received mites during copulation. In most cases, we were able to see that the mites previously on the male's abdomen were on the outside of the female's elytra after the male was removed. In nearly all cases, the number of mites on the female after copulation exactly accounted for the number of mites subsequently observed missing from the parasitized male. This simple result confirms Baker and Eickwort's (1975) observation that *C. labidomerae* is sexually transmitted, like other podapolipids (Hurst et al. 1995). The number and position of mites on the donor male affected the probability of the female becoming parasitized. For each copulation duration, there was a significant association between the number of mites on a male initially, and the number of mites the female received (Fig. 1; Kendall rank correlation: for 60 s, $\tau = 0.689$, $p < 0.001$; for 240 s, $\tau = 0.694$, $p < 0.01$; for 900 s, $\tau = 0.833$, $p < 0.001$). Seven males had no mites visible on the abdomen or dorsal surfaces of the elytra before mating, and six of the seven females that did not receive mites copulated with these males.

There was a significant relationship between copulation duration and the proportion of a male's mites transferred to the female (for median proportion transferred, 60 s: 0.026; 240 s: 0.04; 900 s: 0.176; Kruskal-Wallis, $H = 15.153$, $df = 2$, $p < 0.001$). A greater proportion of mites transferred during the long copulation (900 s) than during either of the shorter copula-

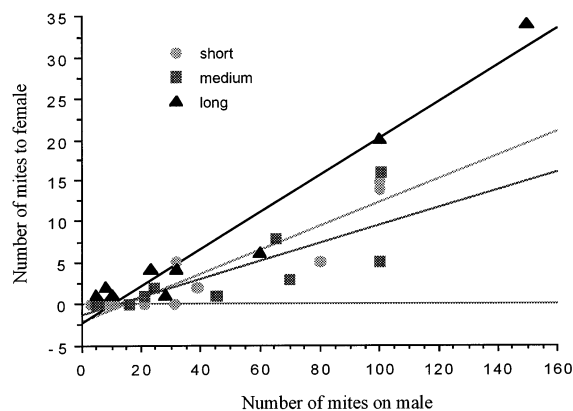


Fig. 1. The number of mites transferred to an unparasitized female from a donor male with a given number of mites, for three different copulation durations. Lines of best fit are shown for illustration.

tions (nonparametric multiple comparisons: for 60 vs 240s, $p > 0.05$; for 60 vs 900s, $Q = 3.55$, $p < 0.001$; for 240 vs 900s, $Q = 3.57$, $p < 0.001$). Thus, the number of parasites a female receives depends in part on mite density on the donor male, the amount of time the two are in contact, and the position of mites at the time of contact.

The experiment on the effect of mites on survival of MLB on a reduced diet was terminated at 230 d, censoring three beetles in the unparasitized group and one beetle in the parasitized group in the survival analysis (i.e., their longevity was only assumed to be equal to or longer than the duration of the experiment). There was a significant effect of mites on female survival (Table 1, Fig. 2; $\chi^2 = 4.94$, $df = 1$, $p < 0.03$) but not on male survival (Table 1, Fig. 2; $\chi^2 = 0.1751$, $df = 1$, $p > 0.6$). There was an average of $46 \text{ mites} \pm 20 \text{ s.d.}$ (range = 10 to 80) on the 15 parasitized beetles which died before the end of the experiment.

In the experiment on the effect of mites on starved males, parasitized males were less likely to survive a two week period without food than were unparasitized males (parasitized group: $n_{\text{alive}} = 6$, $n_{\text{dead}} = 11$; unparasitized group: $n_{\text{alive}} = 17$, $n_{\text{dead}} = 1$; $\chi^2 = 13.58$, $df = 1$, $p = 0.0002$).

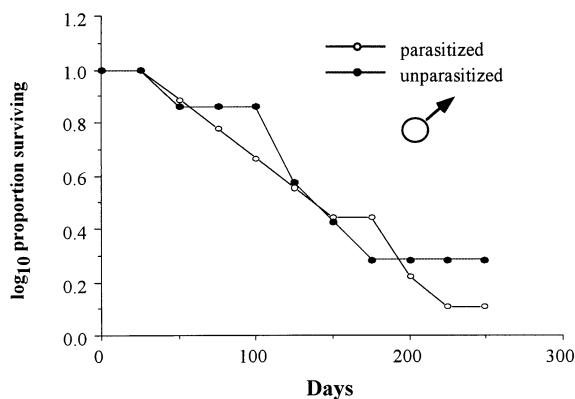
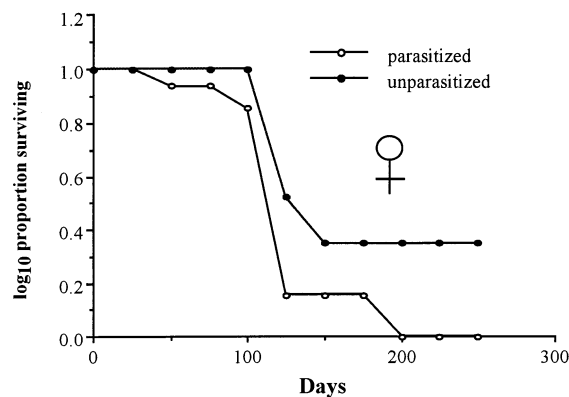


Fig. 2. Survival distribution functions for parasitized and unparasitized female and male milkweed leaf beetles fed at four- to five-day intervals.

Mate choice under risk of parasitism

There was no evidence of a preference for unparasitized mates by either sex. For trials in which both males were contacted (because it is only in these trials that we can compare female behaviour towards both males), females spent a mean time of $222 \text{ s} \pm 29.1 \text{ SE}$ with unparasitized males and a mean time of $195 \text{ s} \pm 24.2 \text{ SE}$ with parasitized males ($t = 0.846$, $df = 26$, $p = 0.40$). There was also no difference in the average number of contacts by females with parasitized ($4.3 \pm 0.47 \text{ SE}$) or unparasitized males ($4.3 \pm 0.43 \text{ SE}$).

With males choosing, males simply mounted the first female contacted in 22 of 36 trials. There was no difference in the frequency with which parasitized and

Table 1. Longevity (d) of parasitized and unparasitized milkweed leaf beetles fed at four- to five-day intervals. Comparisons of survival distributions are by the LIFETEST Procedure (SAS Institute, Inc. 1985)

Sex	Parasitized			Unparasitized			P
	N	Mean (SE)	Median	N	Mean (SE)	Median	
males	9	137 (21.5)	141	7	129 (15.1)	146	n.s.
females	7	108 (16.4)	107	9	128 (6.9)	120	<0.05

Table 2. Summary of frequencies and durations of contacts between parasitized and unparasitized males with unparasitized rival males. Statistical comparisons are between parasitized and unparasitized test males (first and third rows – in bold type).

Treatment group	Male type	<i>N</i>	Average parasite number (SE)	Mean (median) no. contacts initiated	Mean (median) contact duration/trial (s)
parasitized	parasitized test male	30	27.6 ± 6.6	11 (8)*	662 (398)*
	unparasitized rival male	30	n/a	6 (2)	225 (46)
	unparasitized test male	23	n/a	4 (1)	354 (11)
unparasitized	unparasitized rival male	23	n/a	6 (1)	665 (14)

*significant at $p < 0.05$, Mann-Whitney U .

unparasitized females were mounted. In the 27 trials in which males mounted one of the females before the end of the trial (though not necessarily the first one contacted), 14 unparasitized females and 13 parasitized females were mounted.

In the field, a total of 66 males and 52 females were collected. Mite incidence did not differ between copulating pairs ($n = 30$) and singletons: 94% of the single males and 100% of the single females were parasitized, while 96.7% of both males and females captured in copula were parasitized. Unfortunately, many beetles died in transit to the laboratory. The mites also died and desiccated, precluding an accurate count of mites at anything but low densities. Mites were thus scored in categories of greater than 10 or less than or equal to 10. This approximated the median count, as 56% of males and 50% of females that were parasitized had more than 10 mites. By inspection, the burden of mites in this sample appeared similar to that of our laboratory populations, which typically exhibited low median (ca 10–20 mites) and higher mean counts (ca 30–40 mites), and ranged from 1 or 2 mites to > 100 mites per beetle. There was no association between pairs with respect to this measure of parasite number: beetles with small numbers of mites were no more likely to be paired together than with beetles with larger numbers of mites (24 pairs scored; $\chi^2 = 0.178$, $df = 1$, $p > 0.6$).

Male-male competition and parasitism

In the experiment on male competition in which males were paired in the absence of females, contact frequencies and durations with rival males were significantly greater for parasitized test males than for unparasitized test males (Table 2; Mann-Whitney; for contact frequencies, $U = 192$, $p < 0.006$; for contact durations, $U = 230$, $p < 0.04$). There was no difference in the behaviour of rivals towards parasitized and unparasitized test males (Mann-Whitney; for contact frequencies, $U = 308$, $p > 0.5$; for contact durations, $U = 306$, $p > 0.4$). There was no apparent relationship between

the number of mites on the parasitized male and the frequency or duration of contacts initiated toward rivals (Fig. 3; for frequencies, linear regression, ln-transformed parasite number; $r = -0.234$, $n = 30$, $p > 0.2$; for durations, linear regression, ln-transformed parasite number; $r = -0.153$, $n = 30$, $p > 0.4$). In 10 of the 23 trials (44%) in which at least one contact between males occurred, rival males received at least one mite from

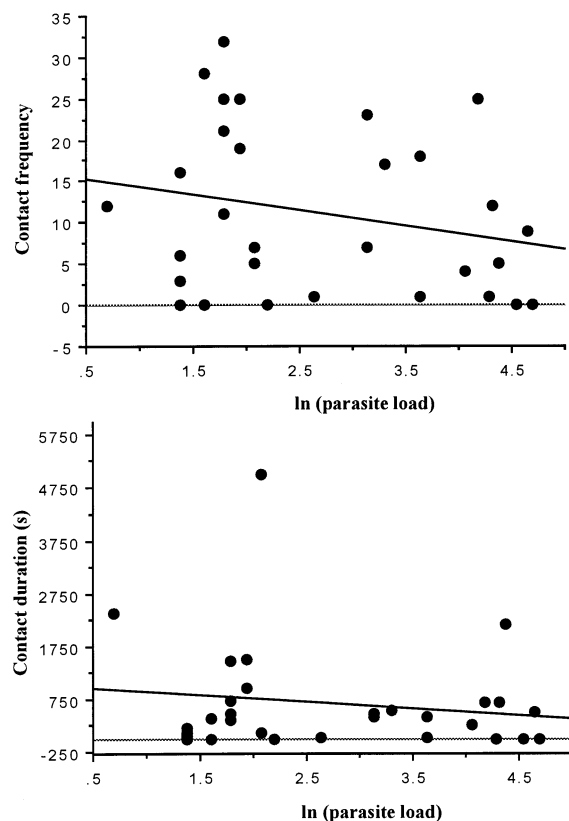


Fig. 3. The contact frequencies ($y = 16.23 - 1.888x$) and cumulative duration of contacts ($y = 1007 - 127.3x$) by parasitized males with unparasitized males in the absence of females, as a function of ln-transformed parasite load.

Table 3. Summary of frequencies and durations of contacts initiated by parasitized and unparasitized males towards rival males in copula with females. Statistical comparisons are between parasitized and unparasitized test males (in bold type).

Treatment group	Male type	N	Average parasite number	Mean (median) no. contacts initiated	Mean (median) contact duration/trial (s)
parasitized	parasitized test male	17	35.2 ± 7.5	5.1 (2)^{ns}	1752 (327)^{ns}
	unparasitized rival male	17	n/a	1.2 (0)	87 (0)
	unparasitized test male	18	n/a	4.2 (2.5)	388 (170)
unparasitized	unparasitized rival male	18	n/a	1.7 (0)	220 (0)

attacking males. There was a positive association between the number of mites on a test male and the number of mites transferred to its rival (trials with no contacts omitted; linear regression, ln-transformed parasite number; $r = 0.549$, $n = 23$, $p < 0.01$). However, there was no relationship between the frequency or duration of contacts and the number of mites transferred to unparasitized males, even though the average, cumulative length of these contacts was substantial ($662 \text{ s} \pm 188 \text{ SE}$).

In the experiment in which test males were paired with copulating beetles, parasitized males displaced rivals in 67% of trials (12 of 18) while unparasitized males displaced rivals in only 33% of trials (6 of 18), a statistically non-significant trend (Fisher's exact test, $n = 36$, $df = 1$, $0.05 < p \leq 0.1$). There was a non-significant tendency for longer contacts to be initiated by parasitized males with copulating males, but no significant difference between parasitized and unparasitized males in the number of contacts with rivals (Table 3). A parasitized male mounted the rival male for the entire trial on one occasion, accounting for the large, but non-significant difference in average, cumulative contact duration.

There was no evidence that unparasitized males avoided parasitized males: contact frequencies and durations of rivals with their test males (parasitized and unparasitized) after take-overs were not significantly different (Mann-Whitney U ; for contact frequencies, $U = 112$, $p > 0.1$; for contact durations, $U = 111$, $p > 0.1$).

In contrast to the experiment with males in the absence of females, there was an apparent dosage effect of mites on male behaviour (Fig. 4). More heavily parasitized males contacted copulating pairs more often (linear regression, ln-transformed parasite number; $r = 0.532$, $n = 17$, $p = 0.03$) and longer ($r = 0.520$, $n = 17$, $p = 0.03$).

In 11 of the 15 trials (73%) in which contact occurred, at least one member of the copulating pair received at least one mite from the parasitized male. There was a significantly positive association between

the number of mites on the test male and the number of mites transferred to its rival (linear regression, ln-transformed parasite number, $r = 0.5$, $n = 15$, $p = 0.04$), but no association between the cumulative duration of contact and the number of mites transferred to the copulating pairs. There were no differences in the number of mites received by males and females.

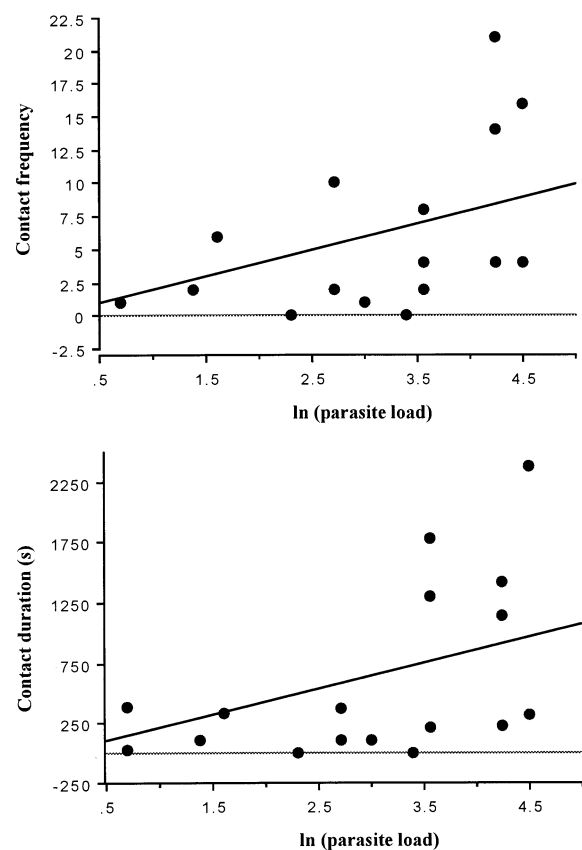


Fig. 4. The contact frequencies ($y = -2.073 + 2.578x$) and cumulative duration of contacts ($y = -276.7 + 293.6x$) by parasitized males with unparasitized males in the presence of females, as a function of ln-transformed parasite load.

Discussion

A large class of traits that ultimately determine the pattern of disease in host populations reflects selection that hosts and parasites impose upon each other's phenotype (Loehle 1995, Thrall et al. 1997, Clayton et al. 1999, Møller et al. 1999). The goal of this study was to explore how sexually transmitted parasites shape host behaviour, in conjunction with the mutual issue of how behaviour shapes the pattern of infection in host populations. Collectively, the series of experiments we performed portray a mildly harmful, sexually transmitted ectoparasite which has a direct effect on the reproductive behaviour of its male host without a corresponding effect on pre-copulatory mate choice. Although we expected *C. labidomerae* to influence MLB mate choice decisions because of its effect on MLB survival and its potential to act as a vector for more debilitating diseases, the relatively low virulence of *C. labidomerae* may explain why there was no such effect. This result is in keeping with the observations of Dickinson (1997), who found little indication of pre-copulatory mate choice in MLBs. The absence of parasite-mediated sexual selection is supported by the frequency of parasitism in the MLB populations we surveyed. By midsummer, over 90% of the Ontario population was parasitized – a prevalence indicative of a host experiencing little or no parasite-mediated sexual selection (Møller 1996).

Although MLBs do not avoid parasitized mates, we measured a direct effect of parasitism on MLB behaviour. In both experiments on male behaviour, parasitized males appeared more competitive than unparasitized counterparts. We detected no behaviours that would indicate a decline in competitiveness. Given the effects of *C. labidomerae* on MLB survival, the increased vigour of parasitized males is unusual. Arguably, a dampening of reproductive vigour is the more typical or 'null' effect, assuming that the principal behavioural by-products of the interaction are mediated via nutritional depletion (Forbes and Baker 1991, Thompson and Kavaliers 1994, Polak and Markow 1995, de Lope et al. 1998, Polak 1998, Thomas et al. 1999).

Because 'winning' struggles with other males clearly can benefit parasitized males, there is the possibility that a heightened vigour is not solely a pathological effect of parasitism, but has a functional basis. Some parasitized organisms, particularly in the early stages of infection, exhibit adaptive plasticity in mating behaviours which mitigate the negative effects of parasitism on reproductive success (e.g., Forbes 1993, Heeb et al. 1998, Richner and Tripet 1999). Recently described examples include egg dumping by parasitized female crickets (Adamo 1999) and elevation of male competitive ability in parasitized fruit flies (Polak and Starmer 1998).

In the case of male MLBs, mating success depends upon the ability to find and monopolize females (Dickinson 1992). Because vagility of parasitized MLBs declines towards the terminal stages of disease (Eickwort and Eickwort 1986), heavily parasitized males should invest relatively more into reproductive effort than unparasitized males or parasitized males in better condition (Forbes 1993, Polak and Starmer 1998). One way to test such a hypothesis is to examine the relationship between parasite burden and reproductive effort, providing that the severity of parasitism in MLBs is a function of the numerical burden of mites, which seems likely. A 'host compensation' hypothesis predicts a positive relationship between parasite burden and reproductive effort, at least in the initial stages of infection (e.g., Polak and Starmer 1998). Male MLBs exhibited significantly positive dosage effects of parasites on competitive vigour in the presence of pairs in copula (Fig. 4), suggesting that more heavily parasitized males were investing relatively more into opportunities to secure mates. Males did not exhibit this dose-dependent effect of mites in the absence of females (Fig. 3), and if anything, the more heavily parasitized males were generally less vigorous than those with a lighter burden (although on average, parasitized males were more vigorous than unparasitized controls). Indeed, that the most heavily parasitized males displayed the most vigour in the presence of females, but not in their absence, suggests an underlying relationship between male reproductive interests and the deleterious effects of parasitism.

In light of what we know of *C. labidomerae* biology, these experiments imply that the changes in male sexual behaviour can enhance opportunities for mite transmission. *C. labidomerae* requires direct contact between hosts for successful transmission, and more frequent and longer contacts can increase the transmission rate (Fig. 1; Drummand et al. 1989). The tendency of male MLBs to increase contact frequency and duration with rivals thus acts in a direction that benefits the mite. We did not confirm, however, this effect in the experiments on male-male competition. We measured no significant association between the duration and frequency of contacts and parasite transmission to either rival males or to females, in contrast to our initial experiment on mite transmission between members of copulating pairs. But this initial experiment also revealed that some of the variation in infection rates is explained by whether mites are already present externally on males. The tendency of *C. labidomerae* to move from beneath the elytra and congregate on the abdomen of male beetles in the presence of females probably explains the contradictory results on mite transmission from the competition experiments (Drummand et al. 1988). In both competition experiments, males attacked each other sporadically, disrupting the continuous transmission of mites we had witnessed in the experiment with isolated

copulating pairs. The mites adjusted to the episodic transmission opportunities by congregating at the precise point of contact between males and females (rather than returning to the subelytral space), waiting there for even fleeting opportunities for transmission. In one trial for example, the parasitized male contacted its rival only once and for no more than two seconds, but the rival male nevertheless received 13 mites.

Because mites can benefit from the change in male behaviour, an obvious alternative explanation for the behaviour of parasitized males is that *C. labidomerae* manipulates male sexual behaviour adaptively in order to increase transmission opportunities (Moore and Gotelli 1990, Møller 1993, Maitland 1994, Poulin et al. 1994, Lockhart et al. 1996, McLachlan 1999). However, our data and what we know of beetle and mite biology together suggest that this interpretation is less likely. For one, the contrasting dosage effects in the two experiments are at odds with a 'parasite manipulation' hypothesis. Even if there were a dose-dependent, 'parasite-adaptive' effect, changes in male vigour that are initiated by mites should occur without respect to the presence or absence of females. It is difficult to construct a scenario (or a facilitating mechanism) in which a positive dosage effect is adaptive for mites only in the presence of females. Secondly, with or without mites, MLBs mate promiscuously and at length (Dickinson 1997), providing ample opportunities to move between hosts. This suggests that there is little scope for conflict between male MLBs and mites over MLB mating behaviour.

In summary, we have considered two components of host mating behaviour, and asked how these might affect parasite transmission opportunities. Our results indicate the absence of sexually selected impediments to parasite transmission in MLBs, and the presence of an effect of parasitism on male competitiveness that can augment opportunities for transmission of a sexually transmitted parasite. To our knowledge, these data provide some of the first concurrent information on host sexual activity and sexually transmitted disease in a controlled setting. Additional work is needed to more fully explore the basis of these changes in male behaviour, and their implications for both MLB fitness and mite transmission in natural arenas.

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