The Effect of the Podapolipid Mite *Chrysomelobia labidomerae* on the Sexual and Social Behaviour of its Host, the Milkweed Leaf Beetle (*Labidomera clivicollis*)

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Abstract

Interactions between animals, such as mating or fighting, provide opportunities for parasite transmission because parasites can move between hosts when they contact each other. For the hosts, one cost of such conspecific interactions may be infection by sexually or socially-transmitted parasites. Unparasitized hosts should thus minimize the probability of infection by avoiding conspecifics infected by contagious parasites. Conversely, variation in host-host contact rates affects parasite fitness because parasites must colonize new hosts in order to persist. By modifying host sexual or social behaviour, parasites can increase their opportunities for transmission. I tested two predictions: that unparasitized hosts should avoid parasitized mates and that parasitized hosts should contact conspecifics more, with the milkweed leaf beetle (MLB), *Labidomera clivicollis*, parasitized by the directly-transmitted, subelytral mite, *Chrysomelobia labidomerae*. *C. labidomerae* colonizes new hosts when hosts contact one another, primarily during copulation or when males contact one another, and feeds on MLB haemolymph. MLBs were reared in the lab and experimentally parasitized with mites. I first established that *C. labidomerae* can be harmful to MLBs. *C. labidomerae* reduces the longevity of female MLBs fed at four to five day intervals, and reduces the chances of a male MLB surviving a two-week period of starvation. Although contacting parasitized mates may be costly, I found no evidence of parasite avoidance either in the laboratory or in the field. However, in the laboratory, parasitized males contact other males more frequently and for longer durations, and are more likely to displace copulating males from females than are unparasitized males. Thus, parasitism by *C. labidomerae* induces a change in male behaviour towards other males in a manner which increases parasite transmission opportunities without inducing an avoidance response in unparasitized hosts. Although there may be alternative explanations for these results, one plausible explanation is that *C. labidomerae* adaptively modifies male behaviour to increase its own transmission opportunities. If so, this represents the first evidence that a sexually-transmitted parasite directly modifies the sexual behaviour of its host.

For Hélène
You've seen it all

For my family, every one of you

Acknowledgments

Because there were times when it seemed to me that I was as likely to successfully complete this degree as were the Gougers to win the "Most Sportsmen-like" trophy (for those of you who don't know, the chances of that - at least before the 1994 season - were vanishingly small), I thought I'd show a complete lack of humility and acknowledge myself first of all for having completed a Master's degree in ecology in Canada. I have wondered from time to time if Canadians, in order to get back at the Great Satan for toxic culture shock, lure gullible Americans north, give them a herculean task called a graduate degree in ecology, and then laugh and laugh at - as Pete Nonacs would say - their little victories. But after seeing Canadian graduate students complete far more difficult research with far more style and accomplishment than I, it's clear that I'm just paranoid, although it's conceivable that even they haven't been let in on the joke.

A friend asked me not to acknowledge him if that implies that he is somehow *responsible.* Thus, although I alone am responsible for this thesis, very little would have been accomplished without the support and assistance of many people. I'll risk naming some, though I'll fail to acknowledge many. For those not mentioned, accept my thanks now.

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Off to Europe I go to thank Børge Damsgøld and Paul and Regula-Schmid Hempel for, at non-overlapping times during this degree, being good friends and mentors. Paul and Regula shared my enthusiasm for Picard and the Borg, among other things. Børge taught me how to cook salmon, to drink Aqua Velva - I mean Aquavit -, and to look at whales in an entirely new light.

Two people in particular deserve their own little space for making this degree an enjoyable experience.

Even though he will always be handicapped by his prairie heritage (he's from Sas**catch**uhwand), Don Hugie was a brave mate aboard the (at times, listing to port) ship of my degree, and steadfastly refused to swim to safety. As he has done for many others in the BERG, faculty and students alike, Don provided many insightful, clear-headed suggestions for the improvement of my research, none of which I acted on. The pride of Tisdale (and nearby farms)!

Alex Fraser is the brains behind the BERG, an extraordinarily nice and good person, a tad weird, a copious consumer of many canned beverages, and a good friend to Mr. Piranha and other teleosts. Alex helped with my research many times and in many ways, and was an invaluable source of information, advice, sanity, and most importantly, good cheer. Couldn't have done it without you, Alvin. There is a place of honor for you in that Big Pub in the Sky with a Golf Course Nearby and a Year-round Hockey Season.

Finally, as it should be, I turn at last to Hélène, who helped me over every hurdle - what better could I offer in appreciation but long, happy days of thanks?

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Chapter 1

General Introduction

"The parasite's world is a small patch of resources surrounded by other small patches where the probability of colonization of any one patch by a parasite or its progeny is very low. In any one patch the resources, being living organisms, are ephemeral, so tenure on a patch is brief. Colonization of new patches carries large risks with potent selection for colonizing ability" (Price, 1980).

Parasites are ubiquitous features of any organisms environment. Increasingly, the field of behavioural ecology has devoted its attention to the roles that parasites and diseases play in shaping the evolution of animal behaviour, and the kinds of animal interactions and characteristics potentially mediated by parasites are diverse, ranging from competition in fish (Milinski, 1985) to group-living in primates (Freeland, 1976) to the evolution of sex (Bell, 1982). Perhaps due to over-long neglect, evolutionary and behavioural ecologists have recently generated an enormous body of theory not only about the adaptive responses of hosts to parasites and diseases, but about the evolution of the parasitic lifestyle as well.

The idea that parasites manipulate the behaviour of their hosts for their own benefit is not new (Holmes and Bethel, 1972). However, as with the evolution of host/parasite interactions in general, it has been only in the past 10 to 15 years that behavioural ecologists have begun to critically examine the evolution of altered host behaviours. The recent proliferation of empirical (e.g. Schmid-Hempel & Schmid-Hempel, 1993) and theoretical (e.g. Lafferty, 1992) studies of the parasite manipulation hypothesis suggests that many more questions about the evolution of parasite-induced changes in host phenotype remain to be asked and answered. These studies are convincingly demonstrating that the reciprocal evolutionary effects of parasites and hosts on each other are far more subtle (Schmid-Hempel & Schmid-Hempel, 1993) and pressing (Williams & Nesse, 1991; Ewald, 1994) than most behavioural ecologists have suspected.

In this thesis I consider the effects of a sexually-transmitted parasite on the behaviour of its host. Since Freeland (1976) and Daly (1978) identified them as a potential cost of mating, empirical and theoretical work suggests that sexually-transmitted parasites may have significant effects on the evolution of mate choice and mating systems (Hamilton, 1990; Sheldon, 1993). Yet, behavioural and evolutionary ecologists still know remarkably little about the prevalence, ecology, or evolution of sexually-transmitted diseases in the wild. There is no comprehensive review of the venereal diseases of wild animals, although a brief survey of the literature of some confirmed or potential venereal diseases suggests that such a review would be profitable (Table 1.1).

Those interested in the role played by parasites in shaping the sexual and social interactions of their hosts normally frame questions from the point of view of the host. For example, a researcher working on sexual selection and sexually-transmitted parasites might typically ask: does female choice function to reduce the probability of acquiring a sexually-transmitted parasite, or is one cost of extra-pair copulations infection by a sexually-transmitted parasite? Although at least one biologist has suggested that it is a question worthy of investigation (Dawkins, 1982), no one has seriously addressed the reciprocal issue of what traits sexually-transmitted parasites possess that increase the rate of transmission between hosts. Do sexually-transmitted parasites adaptively alter the behaviour of their hosts?

It is likely that investigating adaptation in sexually-transmitted parasites will yield insights into the relationship between these parasites and their hosts' behaviour. For sexually-transmitted parasites, the rate of transmission is directly proportional to the frequency of contacts between hosts (Anderson, 1982). Because the frequency of encounters between hosts ultimately depends upon host behaviour, host behaviour thus may limit the transmission rate of parasites, imposing selection on sexually-transmitted parasites to modify host behaviour. Indeed, because parasite-induced changes in host behaviour may be as common as the adaptive responses by hosts that act to minimize the cost of parasitism (Ewald, 1994), investigating manipulation of host behaviour by sexually-transmitted parasites will extend our understanding of the evolution and ecology of these parasites, and perhaps more importantly, will engender further questions about the effect of sexually-transmitted parasites on the behaviour and ecology of their hosts.

The subsequent chapters are organized as follows:

Chapter 2 describes the relevant biological details of my study species, the milkweed leaf beetle (MLB), *Labidomera clivicollis,* parasitized by the subelytral, directly-transmitted mite, *Chrysomelobia labidomerae.* This chapter also describes collecting and laboratory-rearing methods.

Table 1.1 Several suspected or confirmed parasites that move between hosts during sexual interactions. Sheldon (1993) lists 16 suspected or confirmed sexually-transmitted diseases of several species of domestic and wild birds, not listed here. I define a sexually-transmitted parasite as one which uses copulation between hosts as the primary, although not necessarily the only, means of transmission between hosts. In practice, very few sexually-transmitted parasites are restricted to copulation for transmission: intimate contact of any sort may allow transmission.

Parasite	**Host**	**Reference**
Laboulbeniaceous fungus	Carabid beetles	Crowson, 1981
Spilopsyllus cuniculi, rabbit flea	Rabbit (*Sylvilagus* spp)	Rothschild & Ford, 1964
Kennethiella trisetosa, mite	*Ancistrocerus antilope*, wasp	Cowan, 1984
Marine virus	*Feldmannia irregularis*, *F. simplex*, & *Ectocarpus siliculocus*, benthic brown algae	Muller, 1991
Isancistrum subulatae, monogenean	*Alloteuthis subulata*, squid	Llewellyn, 1984
Columbicola columbae & *Campanulotes bidentatus*, chewing lice	*Columbia livia*, rock dove	Clayton, 1990
Pseudodiplorchis americanus, monogenean	*Scaphiopus couchii*, spadefoot toad	Tinsley, 1990
Podapolipid mites	Ecto/endoparasitic on various arthropods	Krantz, 1977
Ustilago violacea, smut fungus	*Dathonia* spp, grasses	Clay, 1991
La Crosse arbovirus	*Aedes triseriatus*, mosquito	Thompson & Beatty, 1977
Nemhelix bakeri, nematode	*Helix aspersa*, snail	Morand, 1993
Oryctonema genitalis, nematode	Dynastide beetles	Poinar, 1983

Chapter 3 describes two experiments I performed on the effect of *C. labidomerae* on MLB survival. Assessing the virulence of *C. labidomerae* is an important first step in determining whether changes in parasitized host behaviour are parasite or host adaptations (Ewald, 1994). Because *C. labidomerae* is easily moved between beetles, and because it was possible to rear MLBs in the laboratory, I was able to test for survival effects of the mites while controlling for correlations between MLB genetic quality and the tendency to acquire mites in the field. Knowledge of the degree of virulence of *C. labidomerae* may also help explain the behaviour of the milkweed leaf beetle towards parasitized mates.

Chapter 4 describes several experiments I performed on avoidance of parasitized mates by MLBs as a potential host defence against acquiring *C. labidomerae*. In order to understand changes in parasitized host behaviour, changes in behaviour by unparasitized hosts must be accounted for as well. More generally, parasite avoidance as a mate choice strategy has received considerable attention by those seeking to explain mate choice patterns (Hamilton, 1990; Sheldon, 1993). Again, because *C. labidomerae* is easy to experimentally move between hosts, the milkweed leaf beetle/*C. labidomerae* association provides a tractable set of species to test the parasite avoidance hypothesis of sexual selection under well-controlled conditions.

In Chapter 5, I describe experiments on the central issue of my research, which was to determine if a sexually-transmitted parasite modifies host sexual or social behaviour in a manner that may increase the transmission rate between hosts. Finally, in Chapter 6, I summarize the results from the previous chapters, and discuss the implications of these results for our understanding of the evolution of sexually-transmitted parasites, their hosts, and host/parasite interactions.

Chapter 2

The Study System and General Methods

The milkweed leaf beetle (MLB), *Labidomera clivicollis* and a subelytral acarine parasite, *Chrysomebolia labidomerae*, comprise the set of species used in this thesis.

The milkweed leaf beetle

The milkweed leaf beetle, a relative of the Colorado potato beetle (*Leptinotarsa decemlineata*) is a large, orange and black Chrysomelid beetle. The milkweed leaf beetle specializes on the milkweed plant (*Asclepias* spp. and *Cynanchum* spp.), and is found throughout the eastern half of the United States and Canada (Baker and Eickwort, 1975; Eickwort, 1977). Although widespread, MLBs typically occur at relatively low densities on their host plants (Dickinson, 1986). *L. clivicollis* has been described as a "rare" species of Chrysomelid (Eickwort, 1977).

L. clivicollis undergoes four larval instars prior to burrowing into the soil for pupation, which usually lasts about three weeks (Baker and Eickwort, 1975; Palmer, 1985). The adults emerge and feed on milkweed for a week or more before ovipositing on the underside of the leaves (Baker and Eickwort, 1975).

Populations of *L. clivicollis* from New York and Ontario experience only one long season (May-September) favorable to reproduction, in which two or three overlapping generations are produced (Baker and Eickwort, 1975; Eickwort, 1977). Mating begins soon after emergence of the overwintered adult in spring or early summer and continues until the following autumn (Dickinson, 1986). MLBs do not defend territories or resources, and their mating system has been described as "scramble competition polygyny", in which the males actively search for females, and intersperse actual copulation with long periods of riding on the female's back (Dickinson, 1992). There is evidence for sperm competition, and increases in the probability of paternity with increased copulation duration. The long periods of association without copulation may represent mate guarding by males (Dickinson, 1992). Males often fight, both in the presence and the absence of females (Dickinson, 1992). When fights occur between a copulating and a solitary male, the copulating male normally "wins" the fight: copulating males are only rarely displaced from females by attacking rivals (Dickinson, 1992). Although fighting with copulating males sometimes results in take-overs, the function of fighting in the absence of females remains unclear. Fighting males may be attempting to toss each other off the plant (Dickinson, 1992).

The Podapolipidae

Chrysomelobia labidomerae (Acari: Tarsonemina: Podapolipidae) is a subelytral beetle parasite first described by Eickwort (1975) on New York state populations of the milkweed leaf beetle, *Labidomera clivicollis*. *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. In all, *C. labidomerae* has been found on seven chrysomelid beetle species.

The podapolipid mites are a family of highly-specialized parasites of arthropod insects, characterized most notably by a reduction in the number of legs in adults of many species; *C. labidomerae* is unique among the podapolipid mites in having four pairs of legs (Eickwort, 1975). The 83 species of mites in the Podapolipidae have been described on insects comprising 4 orders. They range from endoparasites of the trachea of bumble bees and grasshoppers to ectoparasites on cockroaches and to subelytral parasites on many species of Coleoptera (Drummand et al., 1988).

Regenfuss (1972) has described extreme microhabitat specialization of podapolipids parasitizing the subelytral region of carabid beetles. Since the Podapolipidae feed on haemolymph of their host by piercing the cuticle, feeding mites only occur at weakly sclerotized sites under the elytra, such as the intersegmental and pleural membranes and on the wing veins. Because the cuticle thickness and other characteristics differ between these sites, different species of mites are specialized by length of the stylets and other morphology for unique spaces under the elytra. For example, some species of podapolipids with relatively long stylets only feed on veins at the distal end of the flight wings. The cuticle of the host is thick at these sites, and the long stylets of these mites preclude efficient feeding at other sites under the elytra where the cuticle is thinner. An extreme form of specialization is reached in *Dorsipes cryptobius*, a close relative of *Chrysomelobia* spp., which inhabits the thoracic intersegmental fold of its carabid host (Eickwort, 1975). This space is small, and only one female can feed at a time. With one space on each side of the host, only two adult mites can parasitize a host simultaneously. Larval *D. crytobius* feed at the base of the third anal vein on the wing, which also allows room for only one larva on each side.

Although the species that Regenfuss (1972) examined varied in the degree of specialization to sub-elytral spaces on their hosts, across the 27 species of Podapolipidae that were examined, average stylet length increased with the size of the host, likely due to the increase in cuticle thickness with host body size. As well, Regenfuss (1972) found that competition for favorable feeding sites was so intense in many species that only at higher densities would other, less favorable sites be used. In the highly specialized *D. crytobius*, for example, larval mites move to other hosts if the two feeding sites are occupied, even when the prevalence of mites in the population is high. Presumably, it is more beneficial to colonize new hosts in search of unoccupied sites than to feed at less desirable ones.

Life cycle of Chrysomelobia labidomerae

Unlike *D. crytobius, C. labidomerae* utilizes several subelytral spaces under the MLB elytra throughout its life cycle. Like all podapolipids, *C. labidomerae* goes through a three stage life cycle: egg, larva, and adult (Baker and Eickwort, 1975). Eggs are laid on the wings of the host, mostly between the cubital and second anal veins (Fig. 2.1), but at high mite densities, eggs can be found in the metathoracic crevice and along the lateral edge of the abdominal terga (Drummand et al., 1988). Adult females lay on average 1-2 eggs per day, and may lay more than 50 eggs in a month (Baker and Eickwort, 1975).

Larvae mature at the base of the wings or in the crevice between the metathoracic terga of the adult host, where they feed by piercing the cuticle with their stylets (Regenfuss, 1972; Fig. 2.1). The engorged, inactive larvae then migrate to the underside of the elytra, where they develop into adults. Adult females migrate to the abdominal terga or the crevice between the metathoracic terga, where they also feed. Males are found most often on the underside of the elytra where female larvae molt into adults, which can then be inseminated (Baker & Eickwort, 1975). *C. labidomerae*, probably the adult female, overwinters under the elytra of diapausing adult beetles (Baker and Eickwort, 1975; Drummand et al., 1988). Mites apparently synchronize their diapause to match that of their hosts (Drummand et al., 1988).

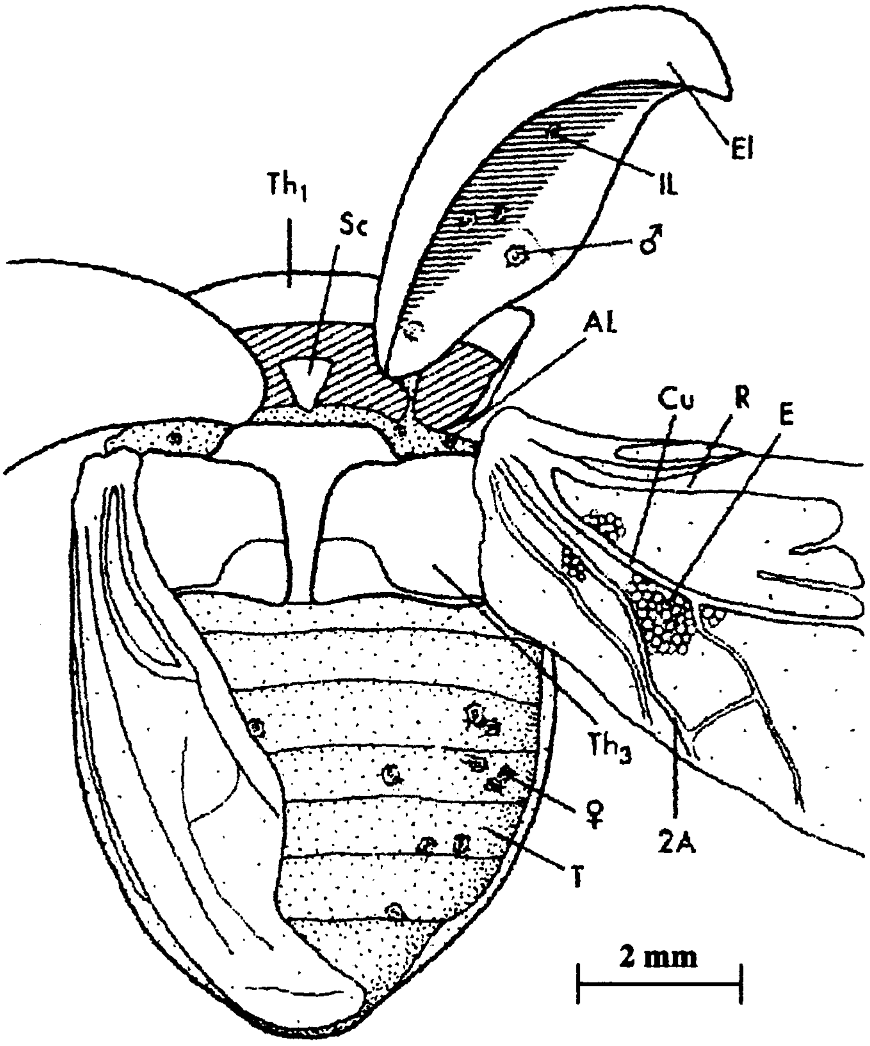

Figure 2.1 Dorsal view of *Labidomera clivicollis* with the wings pulled back to expose *Chrysomelobia labidomerae*. Th1, prothorax; Sc, scutellum; El, elytron; Cu, cubitus; R, radius; 2A, 2nd anal vein; Th3, metathorax; T, abdominal tergum; E, egg; IL, inactive larvae; AL, active larvae (from Eickwort, 1975).

Transmission of C. labidomerae

Only sexually-mature *C. labidomerae* females have the ability to colonize new hosts (Baker and Eickwort, 1975; Drummand et al., 1989). In all other podapolipids, colonization occurs during the larval stage (Baker and Eickwort, 1975). Host-to-host transmission during copulation is a common trait among podapolipid mites, culminating in the extreme specialization of species like *Ovacarus*, which lives entirely within the genital membranes of its beetle hosts and migrates to new hosts during copulation (Stannard & Vaishampayan, 1971).

When diapausing and newly hatched MLB emerge in the spring or early summer, preoviposition female mites normally congregate on the posterior abdominal terga and move to new hosts when the hosts copulate or contact each other (Regenfuss, 1972; Baker and Eickwort, 1975; Drummand et al., 1988; Drummand et al., 1989; pers. obs.), thereby insuring transmission to a new host generation. Transmission occurs from male to female, female to male, male to male when they undergo homosexual courtship or fight, and less often, from female to female. The diploid female mites, if uninseminated, lay haploid eggs on their new hosts, mating with the haploid males to produce diploid daughters (Baker and Eickwort, 1975). (The haplo-diploid status of *C. labidomerae* has not yet been rigorously confirmed, merely inferred from the observation that unfertilized females produce only males and then, weeks later, viable daughters (see Baker & Eickwort, 1975). Haplo-diploidy is common in many families of mites (Price, 1980)).

In the Colorado potato beetle, the mean host colonization rate by preoviposition females increases with the density of mites on the donor (Drummand et al., 1989). However, there is no relationship between mite density and colonization rate by postoviposition females (Drummand et al., 1989). Host age also affects mite transmission: older, moribund beetles carry more mites ventrally, a site advantageous for transmission (Baker and Eickwort, 1975). This age effect occurs even at low mite densities.

Mite population densities

C. labidomerae densities on MLBs are highly variable. Beetles collected from a New York population in 1969 averaged 11 adult female mites per individual, with a maximum of 49 (Baker and Eickwort, 1975). However, some beetles collected in 1993 from an Ontario population yielded infestations in excess of 100 adult mites, although it was common to find beetles with less than 10 mites (Chapter 4; pers. obs.). In a laboratory population at Cornell University, parasite burdens ranged from 4 to 185 adult mites per beetle (Eickwort and Eickwort, 1986), a range similar to that of my laboratory population at Simon Fraser University.

Much of the variance in mite density between hosts may be explained by differences in the duration of infestation (mite numbers increase with time) and differences in the reproductive status of colonizing females (uninseminated females take longer to produce adult daughters because of the extra step of producing haploid sons, hence population growth is slower (Baker and Eickwort, 1975)). *C. labidomerae* populations do not increase geometrically. At higher densities, fecundity is negatively density-dependent: egg production decreases, slowing the rate of population increase (Baker and Eickwort, 1975). This relationship between fecundity and density is consistent with Regenfuss's (1972) study on feeding site specializations. Specialization for unique subelytral spaces, even in the somewhat generalist *C. labidomerae*, puts an upper limit on the rate of population growth on individual beetles. The inability to feed or the inefficiency of feeding at less preferred sites likely explains the decrease in per capita fecundity at higher densities. If so, then the mean fitness of mites decreases at higher densities, an effect that may be useful in understanding the selective environment that has shaped *C. labidomerae* epidemiology and behaviour.

Laboratory populations and methods

Approximately 250 milkweed leaf beetles were collected in June, 1992 from the Queen's University Biological Station at Elgin, Ontario, and in August, 1992 from the Cornell Biological Field Station near Bridgeport, New York. Beetles from both populations were then bred in the lab (thus producing some genetic mixing between populations), producing a laboratory stock of approximately 250 beetles. Beetles were maintained in separate petri dishes with moist dental rolls inserted into a hole in the center of each dish.

All beetles used in experiments were bred in the laboratory. Eggs were collected and reared on moist paper until larvae reached 2nd instar. Larvae were then transferred to large bottles with 5 cm of moist soil at the bottom. Stalks from milkweed plants were inserted into the soil.

At the end of the 4th instar, larvae burrowed into the soil and molted into adults. The entire laboratory stock was maintained at 25 °C with a 16:8 light:dark cycle.

Mites were maintained on designated beetles until needed for experiments. The mite-transferring technique was similar to that used by Eickwort & Baker (1975). The elytra of parasitized beetles were pinned against a cork with "000" pins. Unparasitized beetles were then experimentally parasitized by transferring mites from parasitized beetles to the outside of the elytra with a “000” pin. All unparasitized beetles used in experiments were "mock" infested by touching the elytra of the beetles with a clean "000" pin.

Every one or two days, all beetles were fed fresh milkweed from *Asclepias speciosa* or *Asclepias incarnata* plants grown in a greenhouse at Simon Fraser University. *A. speciosa*, the showy milkweed, was collected near Ashcroft, British Columbia. Although the showy milkweed is not a host of MLB's, beetles readily consumed it. *A. incarnata*, the swamp milkweed, was collected near the Queen’s University Biological Station.

Chapter 3

Reduced Survival of Nutritionally-Stressed Milkweed Leaf Beetles Parasitized by the Mite *Chrysomelobia labidomerae*

Summary

Because parasites remove energy from their hosts, the pathogenic effects of parasites are predicted to depend upon the energy state of their hosts. *Labidomera clivicollis*, the milkweed leaf beetle (MLB), is parasitized by a subelytral mite, *Chrysomelobia labidomerae*. *C. labidomerae* has no significant effect on survival of MLBs fed at two day intervals (Eickwort & Eickwort, 1986). I tested the prediction that *C. labidomerae* are harmful when their hosts are nutritionally-stressed by reducing the amount of food available to parasitized MLBs: one group of parasitized male and female MLBs was fed at four to five day intervals, and another group of males was starved for two weeks. *C. labidomerae* reduced the longevity of female MLBs relative to unparasitized controls fed at four to five day intervals, but had no significant effect on males fed on the same schedule. However, parasitized males were less likely to survive a two-week period of starvation than unparasitized males. These results support the prediction that *C. labidomerae* reduces longevity of nutritionally-stressed MLBs.

Introduction

Parasites acquire nutrients from their hosts, and nutritional deficiencies account for much of the pathology associated with parasitism (Holmes & Zohar, 1990; Crompton, 1991). Thus, the adverse effects of parasites should vary with the nutritional status of their hosts: when food is abundant, for example, some parasites may have little effect on host fitness. The opposite may be true when food is scarce. The inverse relationship between host condition and the pathology associated with parasitism typically may be difficult to demonstrate, perhaps because of the difficulty in manipulating both parasitic infections and host condition (but see Lope et al., 1993).

Chrysomelobia labidomerae (Acari: Podapolipidae) is a subelytral mite parasitizing several species of chrysomelid beetles throughout North America, including the milkweed leaf beetle (MLB, *Labidomera clivicollis*) and the Colorado potato beetle (*Leptinotarsa decemlineata*) (Baker & Eickwort, 1975; Chapter 2). *C. labidomerae* reproduces and matures directly on adult beetles, and adult females move from host to host when hosts copulate (Baker & Eickwort, 1975; Drummand et al., 1989; pers. obs.). Both larval and adult stages of *C. labidomerae* feed on host haemolymph by piercing the abdominal integument of their hosts, and burdens can exceed 100 mites per beetle (Baker & Eickwort, 1975; pers. obs.). By feeding on haemolymph and occurring at high densities, *C. labidomerae* and other podapolipids potentially reduce survival or fecundity of their hosts, and thus may serve as biological control agents of pests like *L. decemlineata* (Eickwort & Eickwort, 1986; Drummand, 1988; Drummand et al., 1989).

Eickwort and Eickwort (1986) measured the effect of *C. labidomerae* on MLBs, finding no effect on fecundity, and a slight, nonsignificant effect on mean survival (days). They concluded that *C. labidomerae*, like other podapolipid mites on other beetle hosts, is relatively non-pathogenic to MLBs. Cantwell et al. (1985) reported similar results for another species of podapolipid mite (*Coccipolipus epilachnae*) parasitizing the Mexican bean beetle (*Epilachna varivestis*), results corroborated by Hochmuth et al. (1987). Schroder (1982), however, had reported a significant effect of *C. epilachnae* on Mexican bean beetle longevity. Moreover, *C. labidomerae* may reduce longevity of the Colorado potato beetle (Drummond et al., 1989).

The available evidence for the effect of podapolipid mites on host survival is thus somewhat equivocal. Different feeding or culturing protocols may explain some of the variation in previous studies on podapolipid pathology. Popapolipid mites like *C. labidomerae*, for example, may have little effect on host longevity when food is abundant. In Eickwort and Eickwort's (1986) study, for example, beetles were fed every other day. Is *C. labidomerae* more harmful when food is less abundant? I tested this idea with laboratory-reared milkweed leaf beetles experimentally parasitized with *C. labidomerae*. I measured the effect of *C. labidomerae* on MLB survival under two different feeding regimes: when MLBs were fed at four to five day intervals, and when male MLBs were starved.

Methods

Experiment 1: The effect of *C. labidomerae* on survival of MLB on a reduced diet

In March 1993, 32 (16 male/16 female) two week old adult virgin MLBs were chosen from a laboratory stock, and half were randomly chosen from each sex and experimentally infested with 20 ± 2 adult female mites. One male and one female beetle were incorrectly parasitized experimentally, thus the parasitized group had seven females and nine males, and the unparasitized group had nine females and seven males. The two groups - parasitized and unparasitized - were matched as closely as possible for weight: (parasitized group = .092 g ± .011 s.d.; unparasitized group =.093 g ± .008. s.d.)

Each beetle was then placed into a 5 cm. diameter petri dish, and all 32 beetles were grouped together into a single tray. The beetles were maintained at 25 °C at a constant light/dark regime (16 light/8 dark) and at high humidity, and were fed equivalent pieces of milkweed (*Asclepias* spp.; approximately 4 cm^2) every four to five days. Because MLBs in the laboratory stock regularly consumed leaf cuttings on a daily basis, but would typically begin to die if starved in excess of 20 days in midsummer (pers. obs.), I reasoned that a four to five day period without food would constitute a reduced diet. These beetles were then observed daily for approximately six months, and any mortalities were noted. Mites were counted on each dead beetle. 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).

Experiment 2: The effect of *C. labidomerae* on starved male MLBs

In May 1993, two months prior to the experiment, 36 previously mated, approximately two month old male MLBs were chosen from the laboratory stock. 18 males were randomly chosen from this group and experimentally infested with 20 ± 2 adult female mites. By visual inspection, males in these two groups were similar in size.

All 36 beetles were maintained in separate petri dishes at 25 °C, a 16 h light: 8 h dark light regime, and high humidity, and were fed *ad libidum* up until the time of the experiment. I then ceased feeding the beetles for 14 days, keeping track of any mortalities that occurred in this period. This experiment was not intended to produce survival distribution functions for the two groups of males, but was designed to be a simple 2 x 2 contingency analysis of survival after two weeks of starvation for parasitized and unparasitized male beetles. The two week starvation period chosen was arbitrary, although the starvation period had to be long enough to challenge the beetles but not so long that all beetles in the experiment died. Reasoning that most beetles, parasitized or not, would die after a month of starvation, I chose approximately half that, and starved the beetles for two weeks.

Results

Experiment 1: The effect of *C. labidomerae* on survival of MLB on reduced diet

The experiment was terminated at 230 days, censoring three beetles in the unparasitized group and one beetle in the parasitized group in the survival analysis. There was a trend towards reduced longevity of the parasitized group (Table 3.1; Fig. 3.1). However, *C. labidomerae* had no significant effect on the longevity of pooled male and female MLBs fed at four to five day intervals (Survival data analysis, SAS Inst.; for Wilcoxon statistics, Chi-square value = 1.8241, df = 1, p = .179).

When the sexes were examined separately, there was a significant effect of mites on female longevity (Table 3.1; Fig. 3.2a; $\chi^2 = 4.94$, df = 1, p = .026) but no effect on male survival (Table 3.1; Fig. 3.2b; $\chi^2 = .1751$, df = 1, p = .676).

There was an average of 46 mites ± 20 s.d. (range = 10 to 80) on the 15 parasitized beetles that died before the end of the experiment.

Experiment 2: The effect of *C. labidomerae* on starved male MLBs

One parasitized beetle died before the beginning of the experiment, and was not included in the analysis. Parasitized males were less likely to survive a two week period of starvation than unparasitized males (Table 3.2; G test, G = 15.205; df = 1; p = .0009).

Table 3.1 Summary of the mean and median longevities in days 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)

	Parasitized			Unparasitized			
Sex	n	Mean (S.E.)	Median	n	Mean (S.E.)	Median	P
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
Total	16	124 (14.1)	111	16	129 (7.4)	123	n.s.

Table 3.2 Contingency table of the effect of *C. labidomerae* on male MLB survival after a two week period of starvation.

Male Type	Alive	Dead
Parasitized	6	11
Unparasitized	17	1

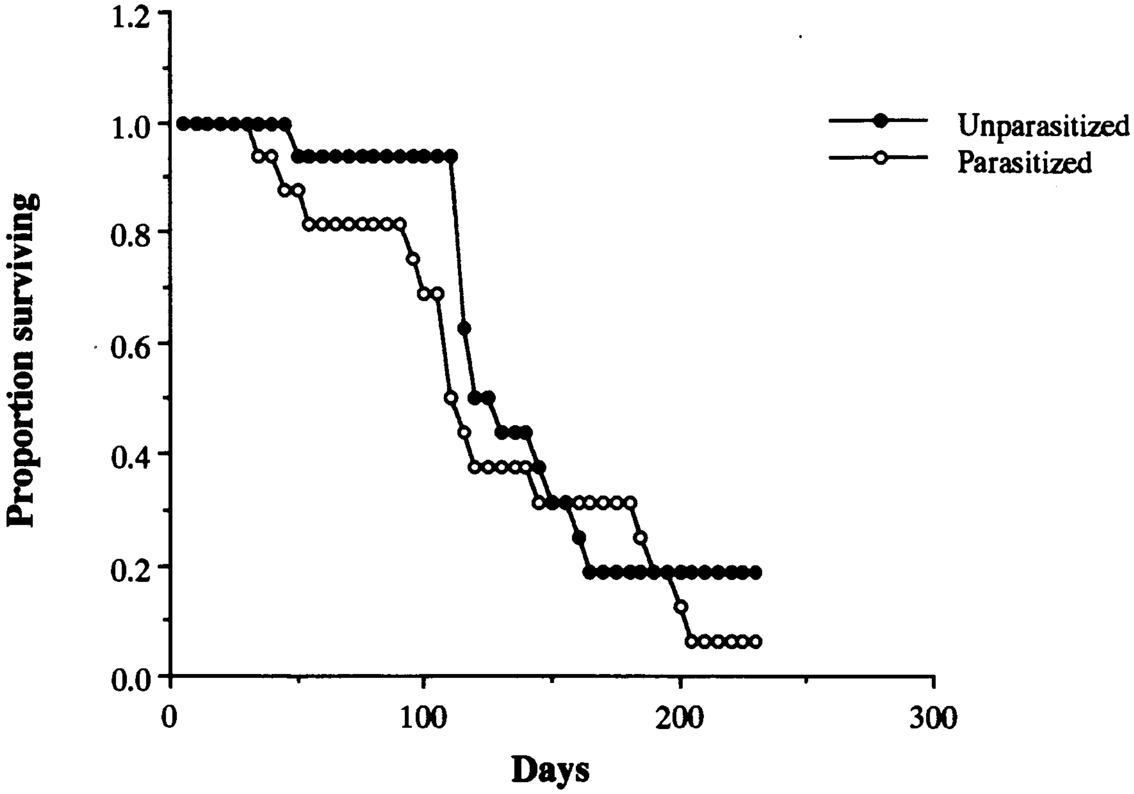

Figure 3.1 Survival distribution functions for parasitized and unparasitized milkweed leaf beetles fed at four to five day intervals. Both groups contain male and female beetles.

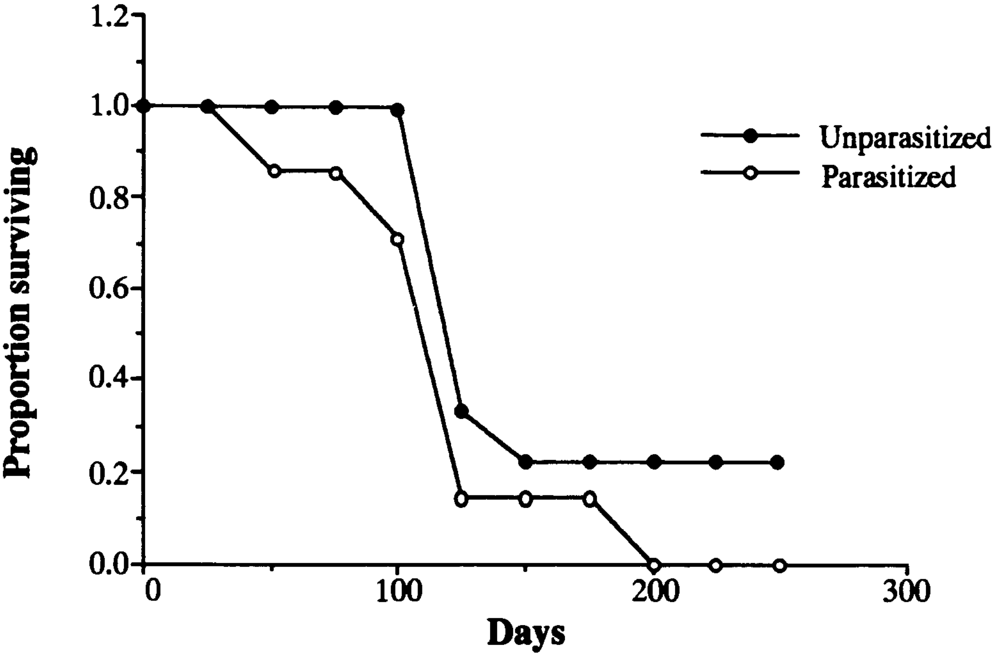

Figure 3.2a Survival distribution functions for parasitized and unparasitized female milkweed leaf beetles fed at four to five day intervals.

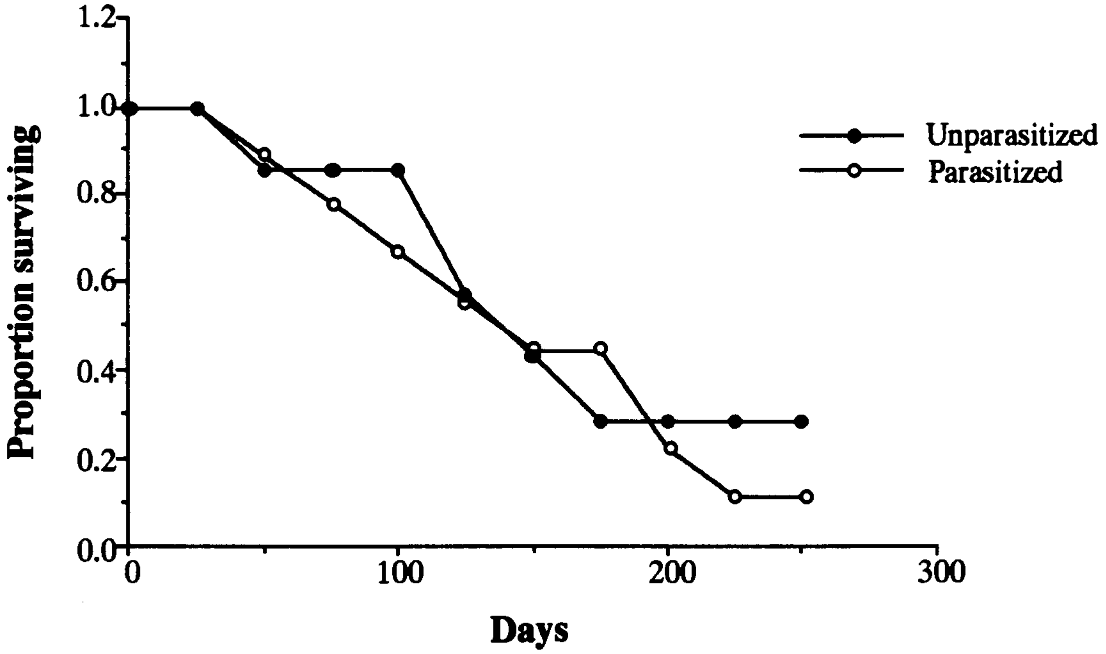

Figure 3.2b Survival distribution functions for parasitized and unparasitized male milkweed leaf beetles fed at four to five day intervals.

Discussion

These experiments tested the prediction that the longevity of parasitized MLBs is reduced when food is relatively scarce. This prediction was confirmed for females fed at four to five day intervals, but not for males. However, starved, parasitized males were less likely to survive a two week period of starvation than unparasitized males. These results confirm and may help to explain Eickwort & Eickwort's (1986) results. Their study demonstrates that *C. labidomerae* may have little effect on MLB longevity when food is relatively abundant. Like Eickwort and Eickwort (1986), I detected a nonsignificant trend towards reduced longevity of parasitized males and females (Fig. 3.1). This trend is mostly explained by the disproportionate effect of mites on female MLBs. Because Eickwort & Eickwort (1986) only report the survival analysis for pooled males and females, it is unclear if there was a similarly greater effect of *C. labidomerae* on females than males.

The asymmetric effect of mites on male and female longevity may reflect a cost of reproduction for females. Because beetles were maintained in separate petri dishes, males expended no energy on reproduction. Females, on the other hand, laid unfertilized eggs throughout the experiment. By competing with their hosts for energy, parasites may increase the costs of energetically-expensive acts like reproduction. Ectoparasites, for example, have been shown to increase the cost of reproduction in swallows (Møller, 1993b).

The starvation experiment demonstrates that survival costs of parasitism are not limited to females. Under relatively more stringent conditions, males are less likely to survive a period of starvation when parasitized. Because males frequently forego feeding to search for females, and are unable to feed during long periods of copulation (Dickinson, 1992), *C. labidomerae* may increase the cost of reproduction for males as well: when parasitized, foregoing feeding for long periods of time may be risky. Future studies manipulating fecundity of female MLBs and mite loads are needed to determine if *C. labidomerae* does indeed increase the cost of reproduction for these beetles.

These results may reopen the debate on the utility of using podapolipid mites as biocontrol agents. During adverse conditions in the field, popapolipids may reduce the longevity of pests like the Colorado potato beetle. Mites like *C. labidomerae* are prolific, hardy parasites, infesting over 95% of some populations of MLB, with the ability to spread rapidly through a host population (Drummand et al., 1989; Chapter 4). Because they would likely be very inexpensive to introduce and maintain in a field population, podapolipids may be useful accessories to other biocontrol efforts against some crop pests.

However, the effect of *C. labidomerae* on MLB survival certainly is not dramatic: the reduction in mean longevity of parasitized females in the laboratory was only 16% of the mean longevity of unparasitized females (Table 3.1). How this would translate to population reduction in the field is uncertain. Current theory on the evolution of parasite virulence suggests that podapolipid mites should be at most only mildly virulent parasites because they ultimately depend on their hosts for their own survival and transmission (see Ewald, 1983). For *C. labidomerae*, which like some other podapolipids, is transmitted during host copulation (Baker & Eickwort, 1975), there may be selection against debilitating the host past the point where the pathology limits transmission. Because mites certainly do not benefit from host death, pathological effects during nutritional stress may simply reflect the outcome of large numbers of mites competing for a dwindling resource (haemolymph). The typical effects of podapolipids on their hosts in the field may then depend upon how commonly food is in short supply for MLBs and other podapolipid hosts.

Regardless of the usefulness of podapolipid mites as biocontrol agents, host/parasite species like the MLB/*C. labidomerae* association described here - in which the hosts are easily reared in captivity and the parasites are typically easy to experimentally-manipulate - may prove to be useful subjects for studying the effect of parasites on life history decisions of their hosts. The activities of most organisms - reproduction, foraging, migration and dispersal, etc. - involve relatively large outlays of energy in energy-limited environments, a circumstance that forms the basis of modern life history theory. There is some evidence, for example, that *C. labidomerae* limits dispersal of Colorado potato beetles (Drummand, 1988). By demonstrating that a chrysomelid beetle is less likely to survive parasitism when energy is limited, these results join the already large body of evidence indicating that parasites have the potential to play significant roles in the evolution of the life histories of their hosts.

Chapter 4

A Chrysomelid Beetle Does Not Avoid Mates Parasitized by Sexually-transmitted Mites

Summary

The parasite avoidance hypothesis (e.g. Clayton, 1990) predicts that animals should minimize the risk of acquiring fitness-reducing pathogens from potential mates by rejecting potential parasitized mates in favor of unparasitized ones. I tested this prediction with *Labidomera clivicollis*, the milkweed leaf beetle (MLB), parasitized by a sexually-transmitted mite, *Chrysomelobia labidomerae*. I found no evidence, in either the laboratory or in the field, to support this prediction. When given a choice between an unparasitized and a parasitized mate, neither male nor female MLBs appeared to prefer unparasitized mates. The results from this experiment were consistent with data from a field survey which detected no difference in infestation rates between mating pairs and singletons gathered from the surrounding foliage. These results suggests the need for caution in invoking parasites to explain mating patterns of their hosts: MLBs do not avoid individuals infected by sexually-transmitted parasites.

Introduction

"There are compelling reasons for believing that animals should not mate indiscriminately, but should choose their mates. Since individuals vary in their quality as potential mates, we would expect natural selection to have favoured mechanisms that ensure that mating occurs with partners of the highest possible quality." (Halliday, 1983)

Given the simple logic of this argument for the evolution of mate choice, we would expect the study of mate choice to be straightforward. However, there is considerable disagreement between those working on sexual selection. Contention most often surrounds those species in which one or both sexes prefer some individuals over others and yet appear to derive no material benefit from the preferred mates. Mate choice in these species has defied general explanation, and the relative importance of the various mechanisms offered as candidate solutions is currently a subject of great debate (Bradbury & Anderson, 1987; Kirkpatrick, 1987a; Balmford & Read, 1991; Harvey & Bradbury, 1991; Kirkpatrick & Ryan, 1991).

Mate choice and parasites

Most examples of non-random mating between conspecifics are the consequence of two forms of sexual selection: intrasexual selection or intersexual selection by mate choice. In intrasexual selection, selection acts on competing members of one sex for access to the members of the opposite sex (Trivers, 1972; Halliday, 1983). Individuals of one sex (usually males) compete and females mate the males successful in competition (Halliday, 1983; Parker, 1983). In "resource-based" female choice (Maynard-Smith, 1991), the female actively chooses her mate on the basis of some direct benefit or resource he provides. Selection occurs directly on the female preference because the benefit the male provides- food or parental care, for example- increases the female's survival or reproduction (Kirkpatrick & Ryan, 1991). Explaining the spread of a preference trait under either of these mechanisms poses little difficulty. Yet, intrasexual selection and "resource-based" female choice fail to explain female preferences in those species in which males provide no resources to the female. Surprisingly, it is in these species where elaborate ornamentation and female choice are often most pronounced (Kirkpatrick & Ryan, 1991).

Proposed mechanisms for the evolution of mating preferences in these species generally fall into two classes: those mediated through direct selection on preferences (costs of searching for mates or sperm fertility differences in males, for example) and those mediated through indirect selection on preferences (Kirkpatrick and Ryan, 1991). Indirect selection mechanisms (Fisher's runaway process or mechanisms based on preferences by females for the "good genes" of males, for example), though theoretically plausible (e.g. Pomiankowski, 1988), have been handicapped by a lack of supporting empirical evidence, as well as failures to always generate falsifiable predictions (Read, 1990; Balmford & Read, 1991; Kirkpatrick & Ryan, 1991).

Explanation of mating patterns in "non-resource based" species has been invigorated by more closely examining the consequences of selection acting directly on preferences, and a careful consideration of what constitute economic benefits (Reynolds & Gross, 1990) or costs (Parker, 1983; Kirkpatrick, 1987b; Real, 1990; Lima & Dill, 1990; Kirkpatrick & Ryan, 1991). Ironically, it was a "good genes" model that helped stimulate this return to considering the direct, non-heritable benefits of choice. In 1982, W. D. Hamilton and Marlene Zuk published a paper in *Science* the topic of which is still controversial and provocative for those working on sexual selection and the evolution of mate choice.

In attempting to resolve a theoretical issue over the maintenance of heritable variation in traits under sexual selection, Hamilton and Zuk (1982) proposed that, because males may vary in heritable resistance to pathogens currently infecting the population, females who choose resistant males would produce more viable offspring than nonchoosers. Showy secondary sexual characters evolved, they suggested, because they were sensitive to the debilitating effects of parasites and were thus revealing signals of the genetic quality of their bearers. Though support for Hamilton & Zuk's original hypothesis is meagre to date (Harvey & Bradbury, 1991; Harvey et al., 1991; see Read, 1990 for a full description of and problems with the original hypothesis), the paper has stimulated a great deal of novel research into the role of parasites as selective agents on host sexual behaviour (Balmford & Read, 1991; reviewed by Read, 1990; McLennan & Brooks, 1991; Harvey & Bradbury, 1991).

Several authors (Freeland, 1976; Daly, 1978; Pomiankowski, 1987; Borgia & Collis, 1990; Clayton, 1990; Hamilton, 1990; Kirkpatrick & Ryan, 1991; Clayton, 1991; Reynolds & Gross, 1990) have proposed that, where parasites are directly contagious to mating partners or their offspring, good genes benefits to mating with parasite-free individuals need not be invoked. If parasite-induced disease is sufficiently costly, females should choose parasite-free males simply to avoid disease. Some heretofore inexplicable preferences in non-resource-based systems may be explained then not by elaborate good genes mechanisms but by inconspicuous - and overlooked - contagious parasites.

The parasite-avoidance hypothesis

These parasite-avoidance models of mate choice predict that, if a potential mate harbours a contagious, infectious parasite which reduces the survival or fecundity of its host, then that mate should be rejected by the choosing sex in favour of an uninfected conspecific. There may be a substantial cost to random mating or mating with multiple partners because of the risk of acquiring parasites, and viability selection should favor mechanisms which minimize the probability of acquiring costly pathogens. Empirical evidence supporting this prediction is accumulating, but is still scanty. Borgia and Collis (1990) provided evidence that satin bowerbirds (*Ptilonorhynchus violaceus*) choose mates in a way that is consistent with parasite-avoidance. Clayton (1990) provided evidence that female rock doves (*Columbia livia*) prefer males with fewer feather lice. More recently, Houde & Torio (1992) confirmed that female guppies (*Poecilia reticulata*) prefer males uninfected by a contagious trematode.

Unfortunately, all of these results are also predicted by Hamilton & Zuk-type good genes models of mate choice (Kirkpatrick & Ryan, 1991). In these studies, females preferred males with fewer parasites and thus more showy secondary sexual characters, exactly as the original Hamilton and Zuk hypothesis would predict. Falsifying the parasite-avoidance model of mate choice requires, in part, testing for choice in a species where there is no discernable effect of parasites on a secondary sexual character. This may seem counter-intuitive - in fact, the parasite-avoidance model of mate choice does not require a revealing ornament as long as parasites are detectable, contrary to Clayton et al. (1992). Because parasite-avoidance does not assume correlations between female choice and males of high genetic quality, parasites need not have any debilitating effects on host phenotype in order for discrimination between mates to occur. Parasite-avoidance models assume only that the choosing sex is interested in whether or not the subsequent mating will result in the transfer of a pathogen. Choice, if expressed, could then be correlated solely with the presence or absence of parasites.

How do females avoid contagious pathogens? Under the parasite avoidance model, selection should favor any behaviour which serves to minimize the cost of mating in the presence of parasites, such as active inspection of mates before mating or post-coital grooming (Hart et al., 1987; Read, 1990; Hamilton, 1990; Clayton, 1991; Sheldon, 1993). Parasites could be detected directly or by their secondary effects like scar tissue, haematomas or even chemo-sensory cues. These "direct indicator traits" are cheat-proof consequences of infection, and can be used by females to assess parasite loads on prospective mates (Read, 1990). Hamilton (1990) speculates that such inspection for direct indicators of parasites may be behind the pre-copulatory cloacal pecking of dunnocks.

I tested the generality of the parasite-avoidance model of mate choice by investigating the mating patterns of a chrysomelid beetle, *Labidomera clivicollis*, the milkweed leaf beetle (MLB), harbouring a sexually-transmitted parasite, the mite *Chrysomelobia labidomerae*. The mite appears to have no discernable visual effect on it's host's phenotype, besides its own occasional presence on it's host's elytra, and is easily moved between hosts in the lab. At relatively high densities, *C. labidomerae* reduces longevity of nutritionally-stressed MLBs (Chapter 3). Thus MLBs may benefit from avoiding parasitized mates.

Here I present the results of two experiments in the lab, and one field study. In the lab, I predicted that unparasitized male and female MLBs should prefer to mate with unparasitized rather than parasitized partners. In the field, I predicted that, if either sex prefers unparasitized mates over parasitized mates, then copulating pairs should have a lower incidence of mites than nearby singletons. Failure to find evidence for preference for unparasitized mates would refute parasite avoidance by active female or male choice in this pair of species.

Mating behaviour of MLBs

In MLBs, males do not hold territories, but simply search for females on milkweed plants, often foregoing feeding to do so (Dickinson, 1992). Once a male encounters a female, there is little or no courtship prior to mating and the male usually attempts to mount immediately (Dickinson, 1986). If he does not do so, the female will often circle the male, touching him with her antennae, occasionally even mounting him to do so (pers. obs.). Females are not receptive to all males (Tower, 1906), often simply falling off the milkweed plant if males attempt to mount (Dickinson, 1992). If males succeed in mounting females, the females may kick the male's legs and aedeagus with their hind legs, turning on their sides in order to jar them off, or prevent intromission by pulling their abdomens under their elytra or keeping their genital openings closed (Dickinson, 1986).

The male does not make a spermatophore, but inseminates the female directly (Dickinson, 1986). Mating is prolonged, likely due to the refractory behaviors by the female. Dickinson (1988) measured copulation duration, and recorded an average of 0.75 ± 0.04 (SE) days with one pair mating as long as 2.5 days. Mating involves periods of actual copulation, interspersed with periods of passive riding on the female's back (Dickinson, 1986). For the male, prolonged mating ensures sperm replacement and also prevents females from mating again prior to oviposition (Thornhill & Alcock, 1983; Dickinson, 1988).

Transmission of Chrysomelobia labidomerae

Chrysomelobia labidomerae is a subelytral parasite of *Labidomera* and *Leptinotarsa* spp. of chrysomelid beetles. The mite feeds on the haemolymph of its host by piercing the soft cuticle with its stylets. It reproduces directly on the host, and has no free-living stage. Only adult female mites are known to move between hosts. Mites normally congregate on the posterior abdominal terga of the parasitized beetle, and migrate to new hosts when the beetle copulates (Baker & Eickwort, 1975; Drummand et al., 1988; pers. obs.). Transmission can occur from males to females, females to males, males to males, and less frequently, females to females (Baker and Eickwort, 1975). Most mites on the exterior of the host beetle migrate within seconds of first contact, but additional transmission occurs as contacts increase in duration (Chapter 5). Mites under the beetle's elytra rapidly emerge when their hosts begin to copulate (Baker and Eickwort, 1975; pers. obs).

Methods

Mate choice experiments

In the laboratory, I conducted dichotomous preference tests for both males and females on an erect Y-maze enclosed in a small test arena (cf. Gwynne, 1982; Fig. 4.1). The erect "Y" was chosen in order to mimic conditions on a real plant. MLBs show strong height-seeking behaviour and the erect "Y" allows beetles to drop from the structure following contact with a member of the opposite sex, indicating rejection of that potential mate.

For male mate choice experiments, 12 unparasitized, virgin females of similar weight were chosen from the laboratory stock: 6 of these were randomly parasitized with 20 ± 2 adult female mites one month prior to the experiment. I attached a small ring made from a '000' insect pin through the posterior region of the left elytra of each female. Females could then be tethered to the ends of the Y- maze by means of a small fishing leader. The maze was washed and dried after each trial. 36 trials were performed, consisting of 36 unparasitized virgin male MLBs of the same age (all eclosed approximately one month before) being randomly presented with the 36 possible combinations of the six unparasitized and six parasitized females. To control for possible side bias, treatment females were alternated between right and left arms of the maze from trial to trial. Following Bateson (1983), choice was defined by the relative amount of time a male spent on either arm of the maze, using lines drawn near each female on both arms, equidistant from the center, as the point of commitment for each male (Fig. 1). Time data were only recorded when males were beyond either line. Prior to these experiments, I noted that contacts often occurred between beetles that did not result in copulation. Since these contacts could be opportunities for mite transmission, I recorded the frequency of contacts by each male with either female, 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 minutes had elapsed.

For female mate choice experiments, I conducted essentially the same experiment with a few minor differences. Reasoning that expression of choice may depend on the female's estimate of available unparasitized mates in the population, I paired 36 unparasitized females individually with unparasitized virgin males for two days, beginning five days prior to the experiment. Each female was then maintained on a milkweed plant (*Asclepias incarnata*) for 24

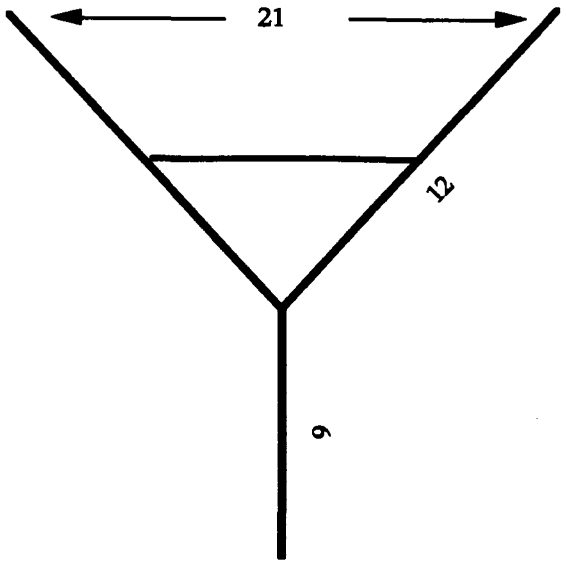

Figure 4.1 Y-maze used for dichotomous mate choice trials. The maze was enclosed in a large box and observations were made through a hole cut into the front of it. The maze stood in a small pan filled with 3 cm. of water. Choosing beetles were introduced to the base of the maze on a floating platform. Test beetles were tethered to the ends of the maze. "Commitment lines" (see text) were 5 cm. from the ends of the arms of the Y-maze. All trials were conducted under flourescent lamps at 25 °C.

hours to ensure that females were well fed before the experiment. I then randomly chose and presented the 36 females to 36 combinations of 12 previously prepared males, 6 of whom were experimentally parasitized with 20 ± 2 adult female mites. I scored each trial for the amount of time spent and the frequency of contacts by females with either male. Each trial ended when females either dropped off the apparatus, or were mounted by a male, or after 15 minutes.

Using paired t-tests, I compared time spent and contacts with unparasitized and parasitized mates only in those trials in which both mates were contacted. I also performed a 2 x 2 contingency analysis for all female pairs on the frequency of being mounted or not mounted by the test male against the presence or absence of mites. The null hypothesis was that the proportions of females mounted for both classes (parasitized/not parasitized) should be equal.

Field study

In order to determine if males and/or females were preferentially choosing unparasitized mates in a wild population, I compared the incidence of mites on copulating pairs with the incidence of mites on singletons of each sex. Two populations of MLB's on *Asclepias syriaca* were sampled at the Queen's University Biological Station (QUBS) by David Bell. From June 9 to June 23, 1993, Bell collected copulating pairs. For each copulating pair collected, two singletons were collected from the nearby vegetation whenever possible. Beetles were placed in marked vials and mailed to Simon Fraser University, where each was scored for the presence or absence of mites. A 2 x 2 contingency analysis was performed between the presence or absence of mites and the mating status (*in copula* or single).

Results

Mate choice experiments

There was no evidence of preference for unparasitized mates by either sex. For trials in which both males were contacted, females spent a mean time of 222 s ± 151 s.d. with unparasitized males and a mean time of 195 s ± 126 s.d. with parasitized males, a nonsignificant difference (Fig 4.2a; t = .84558; df = 26; p = .4055). There was also no difference in the number of contacts by females for trials in which both males were contacted (Fig 4.2b). In 28 of 36 trials, a test male attempted to mount the female, and in 3 trials both males attempted to mount. There was no difference in the rate of mounting attempts by treatment and control males. Test females rejected 40% of the treatment male's mounting attempts and 31% of the control's mounting attempts - a nonsignificant difference.

In the experiment with males choosing, males simply mounted the first female contacted in 22 of 36 trials. Therefore time and contact frequency data show no variance. However, 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. Thus, there was no difference in the frequency with which parasitized and unparasitized females were mounted.

Field study

There was no evidence of differences in mite incidence between copulating pairs and singletons (Table 4.1). 94.4% of the single males and 100% of the single females were parasitized. By comparison, of the paired beetles, 96.7% of both males and females were parasitized. With nearly all beetles parasitized, there was no detectable differences in the presence or absence of mites on copulating vs. single females (G-test; total chi-square = .1; df = 1; p = .8477) or males (G-test; total chi-square = .8; df = 1; p = .9175).

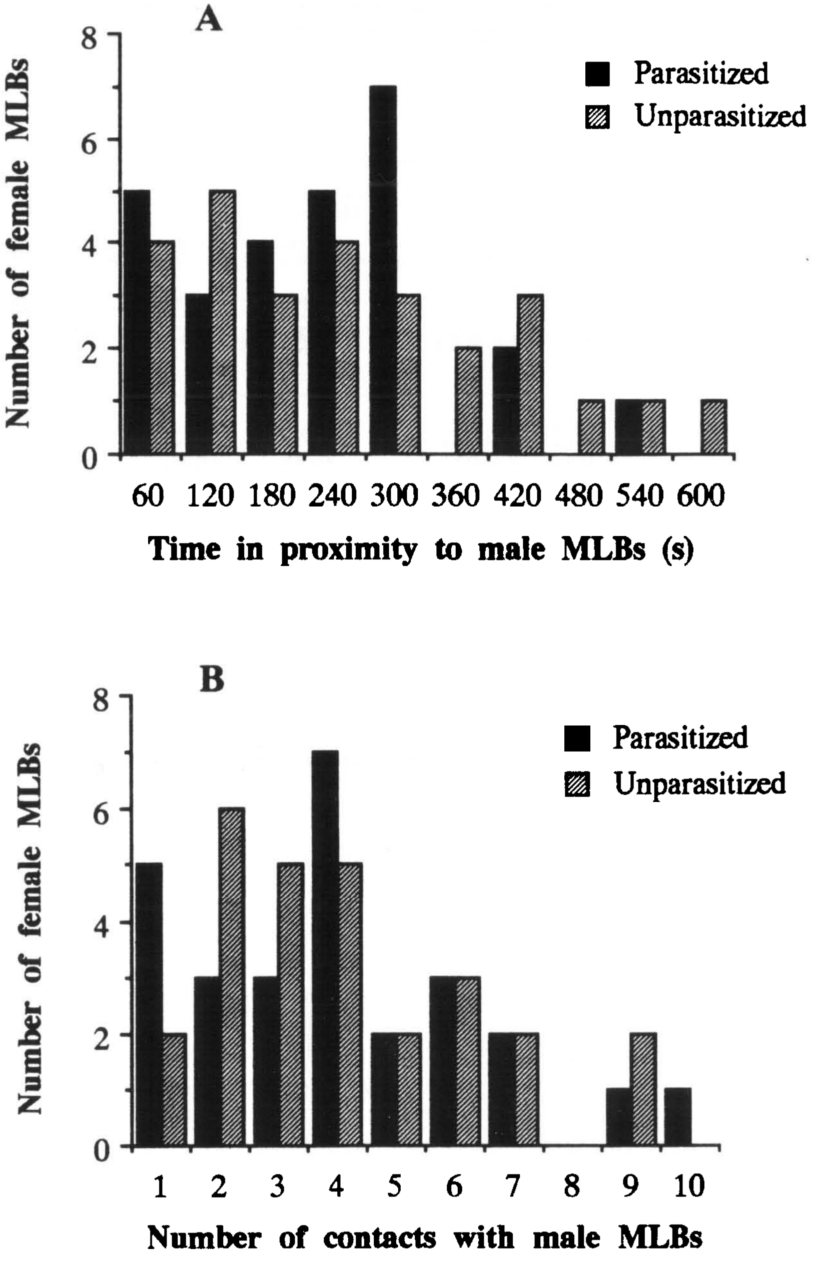

Figure 4.2 Frequency distributions of the results of dichotomous mate choice tests in which females were given a simultaneous choice between an unparasitized and a parasitized male. Only trials in which both males were contacted are reported. Figures represent: a) time spent in proximity to males in seconds; b) number of contacts with males by females (n = 27). Results non-significant at p = .05 (Mann-Whitney U).

Table 4.1 Summary of field data comparing the incidence of mites on copulating pairs versus the incidence of mites on singletons (see text). Numbers for each category are tabulated, and the column percentages are in parentheses.

			Comparisons					
	N	N* para.	> 10 mites	<= 10 mites	Single	Paired	Para. single	Para. paired
Male	66	63	27	35	36	30	34	29
(%)		(95.5)	(43.5)	(56.5)	(54.5)	(45.5)	(94.4)	(96.7)
Female	52	51	24	24	22	30	22	29
(%)		(98.1)	(50)	(50)	(42.3)	(57.7)	(100)	(96.7)
Total	118	114	51	59	58	60	56	58
(%)			(46.4)	(53.5)	(48.4)	(51.6)	(97.7)	(96.7)

*Categories do not sum to the total number of beetles collected in all cases because some beetles were unusable.

Discussion

This study failed to find evidence in the laboratory or in the field for parasite-avoidance in the MLB. Elsewhere (Chapter 3) I show that at high densities *C. labidomerae* reduces the survival of nutritionally-stressed MLBs in the lab. Because the abundance of milkweed plants can be temporally variable (e.g. Palmer, 1985), and thus food shortages may occur in the field, the evidence from Chapter 3 suggests a benefit to parasite avoidance by MLBs. The parasite-avoidance model of mate choice makes the unequivocal prediction of the presence of host adaptations that minimize the probability of acquiring potentially costly parasites from mates. Why then don't MLBs avoid unparasitized conspecifics?

It is possible that there is no benefit to choice because the effect of *C. labidomerae* on MLB fitness in the field is negligible, or perhaps MLBs simply cannot detect the mites on potential mates regardless of selection to do so. If the cost of bearing mites is negligible, then there is little difficulty in explaining these results: there has not been sufficient selection for parasite avoidance. It remains to be determined if either of these possible explanations account for the lack of parasite avoidance by MLBs. However, physiological adaptations to detect mites have evolved in other insect taxa (e.g. Peng et al., 1987), thus it may not be necessary to invoke a constraint on MLB sensory capabilities. Instead, it may well be that the costs of parasitism by *C. labidomerae* are biologically important for MLBs, that the mites are detectable, and that random mating with respect to *C. labidomerae* itself needs explanation.

Where there is variance in the quality of potential mates, typical models of mate choice predict substantial fitness benefits to non-random mating (Janetos, 1980; Parker, 1983; Real, 1990; Roitberg et al., 1992). However, where costs are high relative to the benefits of rejecting the current potential mate, either due to the risk of mortality during searching, the risk of failing to mate, or energetic or time costs, then random mating - i.e., mating with the first mate encountered - may be the dominant strategy over any strategies involving active discrimination between hosts (Parker, 1983; Pomiankowski, 1987; Real, 1990; Maynard-Smith, 1991; Roitberg et al., 1992).

One explanation for the absence of preferences for unparasitized mates is viability selection against choice in the form of costs associated with non-random mating (Pomiankowski, 1987). MLBs clearly mate randomly with respect to mites. Even though there may be some cost to parasitism, and thus some benefit to choice, an alternative strategy of actively discriminating between parasitized and unparasitized mates may offer lower net fitness rewards than random mating because of some unaccounted for cost to choice for MLBs.

In New York populations, MLB's prefer the swamp milkweed, *Asclepias incarnata*, to the common milkweed, *Asclepias syriaca*, even though the latter is far more abundant and is a suitable food source for both larvae and adults (Eickwort, 1977). In controlled experiments, Eickwort (1977) demonstrated that larval mortality is higher on *A. syriaca* than *A. incarnata*, with 54% of the total larval mortality on *A. syriaca* being due to ground-dispersed predators. *A. incarnata*, the swamp milkweed, often grows in moist areas along ditches or ponds, even growing in standing water; *A. syriaca* grows in much drier habitats such as open oldfields. Eickwort speculated that MLBs prefer *A. incarnata* because of a "moat effect": beetles on plants in standing water would be virtually immune to predation by hydrophobic ground-dispersing predators. Even so, she hypothesizes that the low density of MLBs is the direct result of the high overall rates of predation by other insects and birds that she measured in the field; the closely related Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, is notable for it's relatively low rate of mortality due to predation and, perhaps by consequence, it's high densities on host plants.

If the high predation rate on larval MLBs is indicative of the risks to adult beetles, then high search costs may preclude selection for any form of active mate choice by either sex of MLB. Searching for new mates, or avoiding mating attempts by parasitized mates, may involve substantial time in or on risky habitat. Low encounter rates with unparasitized mates (well over 90% of the Ontario population of MLBs were parasitized by mid-June) and high search costs may thus preclude any initial net benefit to parasite avoidance.

Conceivably, the benefit to parasite avoidance by males might be enhanced if, for example, females choose unparasitized males. Parasite avoidance by males then would not only improve survival chances but would improve mating chances as well. Male choice would then similarly enhance the benefit to females of being free of parasites. However, given the low density of MLBs, and the ability of mites to rapidly spread through a MLB population (Baker & Eickwort, 1975), it is difficult to conceive how parasite avoidance could ever get started in the first place. Thus, random mating with respect to mites may be the optimal strategy given low encounter rates and high search costs (Parker, 1983; Real 1990; Roitberg et al., 1992).

For MLBs, even small search costs then may outweigh benefits to choice, a plausible explanation for why MLBs do not avoid parasites with only a moderate effect on beetle longevity. This should be testable: *C. labidomerae* parasitizes seven species of chrysomelid beetles in North America, including two different subspecies of *L. clivicollis*. Predation rates may differ between these species, or even between different populations of the widely dispersed MLBs. If so, then parasite avoidance may well occur in other populations of parasitized chrysomelids with higher host densities and lower rates of predation. A simple first step would be to measure incidence of mites in various populations and species. Highly clumped distributions would suggest, among other things, a low colonization rate of mites between beetles - perhaps evidence of parasite avoidance.

The failure to find of evidence of parasite avoidance by MLBs suggests the need for caution when generalizing about the effect of parasites on the evolution of mate choice. The presence of a directly-transmitted parasite in a host population does not necessarily imply parasite avoidance: population demography and dynamics, biotic and abiotic sources of mortality during searching, cognitive and physiological constraints, the nature of the benefit to choice, and perhaps most crucially the specific life-history traits and transmission strategies of the parasite will collectively determine much of the selective environment of competing mate choice strategies (Real, 1990). Mate choice by parasite-avoidance may not be important, for example, in species without overlapping generations: a truly sexually-transmitted disease could not invade a species without overlapping generations. Directly-transmitted parasites in such systems must have some stage which can persist off the host. If so, then the host may acquire the parasite by many means (as with fleas), thus reducing the direct benefit to avoiding parasitized mates and, if there is practically any cost at all, precluding selection for parasite-avoidance. As well, it may be that even without a free-dispersing stage, directly-transmitted parasites like *Chrysomelobia* may not impose long-term directional selection on preferences for parasite-free mates because of selection on the parasite towards avirulence - a consequence of the trade-off between transmission and host mortality (Ewald, 1983). Finally, when one considers that in a host-parasite system selection acts on two species sharing the same resources, and often with fundamental conflicts over host decision-making (Holmes & Bethel, 1972), ignoring the direct, physiological effect of parasites on their hosts may fail to account for the true complexity of host sexual behaviour (Chapter 5). These latter points have received little attention by researchers working on parasites and the evolution of mating preferences, but may ultimately yield significant explanatory power for those seeking to understand the role of parasites in sexual selection.

Chapter 5

Evidence for Alteration of Host Behaviour by a Sexually-transmitted Mite (*Chrysomelobia labidomerae*).

Summary

Parasites can modify host behaviour to increase the probability that their host contacts another host, thus increasing opportunities for their own transmission. Because sexually-transmitted parasites move directly between hosts during the sexual and social interactions of their hosts, variation in sexual and social behaviour can affect the fitness of these parasites. Thus, sexually-transmitted parasites may be selected to modify host sexual and social behaviour. I tested the prediction that the sexually-transmitted mite, *Chrysomelobia labidomerae*, will increase transmission opportunities by modifying the behaviour of its host, the milkweed leaf beetle, *Labidomera clivicollis* (MLB). I predicted that parasitized male MLBs will contact conspecifics more than will unparasitized males. Two separate experiments support this prediction by showing that parasitized MLBs behave in a way that appears to increase *C. labidomerae* transmission opportunities. In the first experiment, parasitized male MLBs were more likely to displace rivals from females than were unparasitized controls and, in doing so, contacted these rival males for longer durations. In the second experiment, with two males placed together, parasitized males contacted unparasitized males significantly more and for longer durations than did control males. In a third experiment, I showed that longer (as opposed to more) contacts may also benefit *C. labidomerae* because proportionally more mites migrate when beetles are in contact longer. Although there may be alternative explanations for these results, the changes in MLB behaviour are consistent with what would be expected if these changes represented adaptive modification of host behaviour by *C. labidomerae*.

Introduction

"[I]t is important to note that...*behaviour patterns* have a major effect on disease transmission. They will determine both the frequency and the intimacy of contact between individuals." (Anderson, 1982).

Transmission between hosts is a critical component of the reproductive success of parasites. At the minimum, each parasite must generate at least one new infection in order for a parasite population to persist in a host population (May, 1991). For many parasites - those that reproduce directly on their hosts, for example - the most evolutionarily significant measure of reproductive success may be the number of new hosts infected in a population of susceptible hosts (Anderson, 1982). Because rate of colonization of new hosts is determined in part by the number of transmission opportunities available to a parasite, reproductive success is inextricably linked to the chances of a susceptible host contacting an infected host or vector. Ultimately, the probability that a susceptible host contacts an infected host or vector depends upon host behaviour, and parasites can increase the chances that their host contacts a susceptible host by modifying the host's behaviour (Moore & Gotelli, 1990).

Although there is not yet a consensus among evolutionary biologists about the generality of the phenomenon (compare Ewald (1994) with Yan et al. (1994) and references therein), parasite-induced changes in host behaviour that appear to benefit the parasite are well-described outcomes of host/parasite interactions (Holmes & Bethel, 1972; Smith-Trial, 1980; Hurd, 1990; Moore & Gotelli, 1990; Keymer & Read, 1991). Under the parasite manipulation hypothesis, symptoms of parasitic infection are (or are associated with) parasite adaptations that function to increase the rate of parasitic transmission: the diarrhea in humans induced by the bacterium that causes cholera, or the behavioural changes induced in the isopod *Armadillidium vulgare* by an acanthocephalan parasite that make the isopod more vulnerable to starlings (*Sturnus vulgaris*) are two of the diverse examples of parasites modifying the behaviour or physiology of their hosts in order to increase the probability of transmission to new hosts (Ewald, 1994; Moore, 1983).

The vast majority of altered behaviours that have been described involve parasites which move between definitive hosts with the aid of one, sometimes two or three, intermediate hosts (Dobson, 1988). These indirect life-cycle parasites modify the behaviour of intermediate hosts to increase the probability of contact with the definitive hosts. By contrast, directly-transmitted parasites - those that use only one host species - rarely have been considered in studies of altered host behaviours, although there is evidence suggesting that direct life-cycle parasites modify host behaviour (Dobson, 1988; Moore & Gotelli, 1990; Møller, 1993a). Dobson (1988), for example, suggests that the increased activity and aggression associated with rabies is a parasite adaptation for increasing transmission rates.

Modification of prey behaviour by indirect life-cycle parasites is of particular interest because of the implications these changes in behaviour have for predator/prey interactions and population dynamics (Dobson, 1988; Hudson et al., 1992; Lafferty, 1992). Similarly, to the extent that social interactions are avenues for disease transmission, the lack of scrutiny of direct life-cycle parasites is remarkable considering their potential effect on the social behaviour of their hosts (Freeland, 1976; Hamilton, 1990). Variation in host social behaviour undoubtedly affects the chances of infected hosts contacting uninfected hosts (Anderson, 1982; Keymer & Read, 1991), and thus social behaviour can directly influence parasite reproductive success by affecting the opportunities for parasite transmission. Direct life-cycle parasites could, in theory, modify host social behaviour to increase the opportunities for transmission.

Sexually-transmitted parasites are one class of directly-transmitted parasites that require direct host contact for transmission (as opposed to those directly-transmitted parasites that use vectors like air or water for transmission). Even though there is a direct and positive relationship between host sexual interactions and the opportunities for parasite transmission, evolutionary biologists have virtually ignored sexually-transmitted parasites in studies of altered host behaviours (Chapter 1; see Keymer & Read's (1991) discussion of the impact of parasites on social behaviour).

Modification of host behaviour by sexually-transmitted parasites may result from conflicts of interests between these parasites and their hosts over host sexual or social behaviour. The capacity of sexually-transmitted parasites to reproduce and spread through a susceptible population probably exceeds the actual opportunities for transmission offered by host sexual interactions. Thus, almost any aspect of host sexual behaviour is potentially a source of conflict between the genetic interests of sexually-transmitted parasites and their hosts.

However, as with any altered host behaviour, the evolution of a parasite trait which modifies host sexual behaviour depends in part upon the cost of such a trait relative to the benefit gained from the altered behaviour. We might expect that even at low costs to the parasite the net fitness benefit of modifying some aspects of host sexual behaviour would be small. To what extent, for example, do we expect males in polygynous mating systems to limit the number of copulations with females to some level below that to which it might be raised by parasite-induced changes in behaviour?

Any parasite-induced increase in copulation rate may reduce male survival (due perhaps to predation risk or reduced foraging opportunities), and because sexually-transmitted parasites depend on their host's survival for their own survival, compromising host survival rate may ultimately reduce the benefit the parasite accrues from altering copulation rate (Dawkins, 1982; Anderson & May, 1982; Ewald, 1983).

Any hypothesis about a change in host sexual behaviour induced by a sexually-transmitted parasite must thus target behaviours where there may be significant fitness differences between the host optimum and parasite optimum. For example, Segura et al. (1988) found that the ejaculate volume of male white leghorn chickens infected by a strain of a venerally-transmitted lymphoid leukosis virus was significantly greater than that of controls. Although Segura et al. (1988) offer no ultimate explanation for this result, we might imagine that a venereal parasite that moves from males to females in the seminal fluids may gain a significant benefit from large seminal volumes: more parasites are transmitted in larger ejaculates. Male leghorns, on the other hand, may be expected to optimize the amount of semen necessary to achieve paternity, given that semen production may be costly. Such an interpretation is strengthened by Segura et al.'s (1988) observation that the same viral strains reduce the fertility of male chickens. Clearly, reduced fertility is not in the best interests of male leghorns. Ejaculate volume may be a significant area of conflict between parasites transmitted by semen and their hosts.

Here I describe tests for such conflicts of interest between sexually-transmitted parasites and their hosts by investigating changes in behaviour of parasitized milkweed leaf beetles (MLB) (*Labidomera clivicollis*). Male MLBs occasionally fight one another (Dickinson, 1992) or engage in "homosexual courtship" (Baker & Eickwort, 1975). Fights sometimes occur between a copulating male and a solitary male, and presumably these fights are over access to the female (Dickinson, 1992; Chapter 2). Copulating males are only rarely displaced as a result of these fights. Males also fight in the absence of females, but the function of these fights or of male-male courtships remains to be determined (Dickinson, 1992). Males are not territorial, and their mating system has been described by Dickinson (1992) as scramble-competition polygyny, because males actively search for and monopolize females, assuring paternity by guarding females until oviposition.

The sexually-transmitted, subelytral mite *Chrysomelobia labidomerae* parasitizing *L. clivicollis* can move between males when they fight or engage in homosexual courtship (Baker & Eickwort, 1975; Chapter 2). I argue that a parasite-induced elevation in male MLB activity or aggression, manifested as an increase in fighting or homosexual courtship between males, will increase the chances a host beetle contacts another beetle. Because contacts between male beetles represent transmission opportunities, *C. labidomerae* can increase the probability of transmission from a male host by modifying the rate at which he fights or courts (and thus contacts) other males. Male-male contacts may be a significant area of conflict between host and parasite optima. Unparasitized male beetles may limit fighting or courtship for the same reasons that non-rabid animals presumably limit activity or aggression: elevated activity or aggression must entail some costs, due to energetic limitations, risk of predation, risk of injury, or perhaps most relevant to male MLBs, lost mating opportunities.

I describe two experiments designed to detect changes in contact rates or contact durations between parasitized male MLBs and either unparasitized copulating pairs or unparasitized solitary males. In both cases I predict that parasitized males should contact unparasitized beetles more and for longer, arguing that such results would constitute evidence of modification of male behaviour by the sexually-transmitted mite, *C. labidomerae*, to improve the chances of transmission from parasitized males. I describe one further experiment that quantifies the relationship between contact duration and the number of mites transferred from a parasitized male to an unparasitized female, to test the prediction that unparasitized females receive proportionally more mites during longer contacts.

Methods

Experiment 1: The effect of mites on male contacts with copulating pairs

From 18 July, 1993, to 23 July, 1993, I conducted a laboratory experiment in which I measured the number and duration of contacts by laboratory-reared parasitized and unparasitized males with copulating pairs.

Approximately six weeks before the experiment began, 36 test males were randomly chosen from the laboratory stock, and half were experimentally parasitized with 20 ± 2 adult female mites. The average weights of 18 parasitized and 18 unparasitized males were 0.103 g ± 0.013 s. d. and 0.104 g ± 0.017 s. d., respectively.

To establish copulating pairs, I placed 36 unparasitized virgin males with 36 unparasitized virgin females in separate 5-cm diameter petri dishes and allowed them to copulate. Pairs were matched as closely as possible by size, although no quantitative measure was used (Dickinson (1988) found no evidence of size assortative mating, and no evidence that smaller males attack larger males less often than reciprocally). By visual inspection, sizes of the beetles comprising copulating pairs were very similar to test male sizes. Copulating pairs were marked with liquid correction fluid on their elytra in order to keep track of the three beetles in each dish. Marking was done at least six hours before the beginning of each trial. Liquid correction fluid did not appear to have any effect on beetle behaviour, and was easily removed from the elytra after each trial. Because beetles are most active at night (Dickinson, 1988; pers. obs.), all trials were started between 1900 and 2000 h in a darkened laboratory and filmed under infrared light. Test beetles were removed from experimental dishes the morning following each trial.

All beetles were scored for mites at the end of the experiment, and the total number of adult female mites originally on the parasitized male was determined by adding up all mites on all beetles.

Six trials were conducted a day. Approximately one hour after the beetles were paired, I introduced a test male to each petri dish. Test males had been randomly assigned numbers by an assistant, and I scored each trial without knowing if the test male was parasitized or not. Trials were taped for six hours, and I later scored each dish for: (1) the number of contacts by the test male with the rival male, and (2) the duration of each contact by the test male with the rival male. Because one manifestation of parasite-induced increases in activity or aggression might be a change in the rate at which the copulating males are displaced from females, I also recorded: (3) the success or failure of each contact in displacing the rival male when the rival male was copulating, and (4) given success in displacing the rival male, whether or not the test male attempted to copulate with the unoccupied female. Finally, I scored (5) contact rates and durations with test males by the rival males in each trial.

Contacts were defined as any initiated contact between beetles that lasted longer than three seconds. A "three second rule" was used only because of occasional difficulty in discriminating proximity in a small petri dish from the behavioural act of contacting another beetle. Any contacts lasting longer than three seconds were unequivocal.

Although the purpose of this experiment was to measure the effect of mites on contact behaviour of their current hosts, this experiment was potentially confounded by avoidance of parasitized males by the unparasitized, rival males (there is no reason to suggest that unparasitized males would be more rather than less likely to contact parasitized males). This confounding factor is conservative, however, as avoidance of parasitized males would have the effect of decreasing the number of times beetles came into contact, thus reducing the likelihood of finding an increase in contact frequency and duration relative to controls. Nevertheless, rival male behaviour was closely monitored in order to detect any avoidance behaviour.

Mann-Whitney U tests were used to determine if parasitized males contacted rival males longer and more often or, conversely, if rival males initiated contact with parasitized males less often or for shorter durations. Except where first contact durations are compared, contact durations by the parasitized and the unparasitized males represent the cumulative durations per trial. G-tests with William's correction were used to determine if the rival males in the trials with parasitized males were more likely than the rival males in trials with unparasitized males to be displaced at least once.

To determine if there is a relationship between parasite loads and contact behaviour, I used Spearman rank correlation to compare the number of mites originally on parasitized males with the duration and frequency of contacts by parasitized males.

Experiment 2: The effect of mites on male contacts with unparasitized solitary males

I measured the frequency and the duration of contacts by laboratory-reared parasitized males with laboratory-reared unparasitized solitary males in the laboratory from 3 August to 6 September, 1993.

The experiment involved four groups of male beetles: (1) 30 parasitized, "test" males paired with (2) 30 unparasitized males, and (3) 23 unparasitized "test" males paired with (4) 23 unparasitized males. I 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". I refer to the two males in each trial as the "test" male (parasitized or unparasitized) or the "rival" male (unparasitized). Sample sizes were unequal because of a limitation on the number of available male beetles of the same age and condition in the laboratory stock.

106 virgin male beetles were chosen from the laboratory stock and 30 were randomly chosen from this group and experimentally parasitized by 20 ± 2 adult female mites. The remaining beetles were "mock" parasitized. By visual inspection alone, as above to minimize handling of the beetles, all males were very similar in size to each other.

Seven to eight trials were conducted a day in 5-cm diameter plastic petri dishes. In the parasitized group, parasitized and unparasitized males were paired at random (n = 30), and in the control group, 23 unparasitized male beetles were randomly chosen to be test males and assigned an unparasitized, rival male. To facilitate analysis, one beetle in each dish was randomly chosen and marked with correction fluid on an elytron. As in the previous experiment, this did not appear to have any effect on beetle behaviour. I analyzed all trials without knowing which of the two males in each were test males.

In each trial, for two hours I scored: (1) the frequency of contacts initiated by the test male (the parasitized male in the parasitized group and the pre-selected male in the control group) with the rival male; (2) the duration of each contact initiated by the test male with the rival male; (3) the frequency of contacts initiated by the rival male with the test male; (4) the duration of each contact initiated by the rival male with the test male. Contacts were defined as above.

The purpose of this experiment was to determine the effect of mites on their male host's behaviour towards a solitary, unparasitized male. As in the above experiment, interpretation of the results is potentially confounded by the effect of mites on the behaviour of the rival male. Rival male behaviour towards the parasitized males was closely monitored in order to detect avoidance.

Comparisons of the contact frequencies and durations of the 30 parasitized males with their rivals with the contact frequencies and durations of the 23 test males with their rivals were then carried out (Mann Whitney U) to determine if there was a positive effect of mites on current host behaviour. As above, except where first contact durations are compared, contact durations are the cumulative durations per trial.

As in the previous experiment, I correlated (using Spearman rank correlation) the number of mites originally on parasitized males with the duration and frequency of contacts by parasitized males in order to determine if there was a relationship between parasite loads and contact behaviour.

Experiment 3: The effect of copulation duration on mite transmission

In order to quantify the relationship between contact duration and mite transmission, I measured the number of mites transmitted from male to female laboratory-reared beetles for three different copulation durations.

Three groups of parasitized males were mated with females for three different durations: 60 (n = 10), 240 (n = 9), and 900 (n = 9) s. Male size was matched by visual inspection, and mean mite numbers were 42.5 ± 11.8, 49.8 ± 12.0, and 46.2 ± 16.4 for the 60, 240, and 900 s copulation groups, respectively. The three copulation durations were chosen arbitrarily, although I tried to select durations which spanned those occurring in the previous experiments.

Females rather than males were selected as partners for the parasitized males because of experimental tractability: males will nearly always mate with females given the opportunity, but court other males far less often. As the results of the previous experiments (described below) indicate, there is no reason to suspect that the transmission rate from males to females is qualitatively different from the transmission rate from males to other males, nor is there evidence from the field collections that *C. labidomerae* prefers one sex over the other (pers. obs. - Chapter 4).

A female was introduced to the 5-cm petri dish containing each male, and males were allowed to copulate for the designated period and then removed from the female. Females were then left overnight, and scored the following day for mites. All petri dishes were thoroughly checked under a light microscope for displaced mites. In nearly all cases, the number of mites on the female after copulation exactly accounted for the number of mites missing from the parasitized male.

I used a Kruskal-Wallis test to determine if the percentage of mites transmitted from parasitized males to unparasitized females were the same for the three copulation durations. I then performed nonparametric multiple comparisons to determine which copulation durations were different.

Results

Experiment 1: The effect of mites on male contacts with copulating pairs

One trial was removed from analysis of contact durations and frequencies because the parasitized male mounted the rival male for the entire six hour trial (Dixon's test for outliers, $p < 0.05$; Sokal & Rohlf, 1981). Removal of this trial from analysis is conservative, and has no qualitative effect on the results of this experiment.

On average, parasitized males tended to contact rivals slightly more than did unparasitized males and, over the six hour trial, did so for longer, although both trends were nonsignificant (Table 5.1). However, a post-hoc analysis (see Discussion) of the first contact durations with rival males revealed that first contact durations initiated by parasitized males were significantly longer than the first contacts initiated by unparasitized males (Mann Whitney, $U = 66.5$, $p = .0344$).

Parasitized males were more likely to displace rival males from females at least once in a six hour trial than unparasitized males ($G = 3.91$, $n = 18$, $p = .047$). Parasitized males displaced rivals in 67% of trials (12 of 18) while unparasitized males displaced rivals in only 33% of trials (6 of 18). This difference in displacement rates was not due to avoidance of parasitized males by their rivals. When parasitized males copulated with the female after displacing the rival male, the rival male initiated contact with the parasitized male in all 8 of 8 trials. When control males displaced the rival male and attempted to copulate with the female, rival males initiated contact in 3 of 5 trials (60%). Although sample sizes are small, there is clearly no trend towards lower contact rates with parasitized males. As well, comparisons of contact durations and frequencies reveal no significant evidence of avoidance of parasitized males by rivals. Rivals initiated contact with parasitized males no less often than they initiated contact with unparasitized males (Table 5.1).

There was no significant difference and no trend in the propensity to copulate with the female once the rival male had been displaced. Parasitized males mounted and copulated with females in 67% of the 12 trials in which they displaced the rival males at least once while unparasitized males mounted and copulated with females in 83% of trials in which this was possible (5 of 6 trials). Over all trials, parasitized males copulated with females in 44% of

Table 5.1 Summary of contact frequencies and contact durations initiated by one male towards the other in an experiment designed to measure male aggression towards copulating males over a six hour period. Comparisons are between parasitized and unparasitized test males (in bold type). Medians in parentheses. N's for first contact durations are for those trials in which contact occurred at least once.

Male type	N	Avg. # contacts initiated	Avg. contact duration/ trial (s)	Avg. duration/first contact (s)
Parasitized test male	17	**5.1 (2)**$^{\text{ns}}$	**603(315)**$^{\text{ns}}$	**448 (104)*** (n = 16)
Rival male[a]	17	1.2 (0)	87 (0)	--
Unparasitized test male	18	**4.2 (2.5)**	**388 (170)**	**127 (50)** (n = 15)
Rival male[b]	18	1.7 (0)	220 (0)	--

[a] denotes rival males paired with parasitized males

[b] denotes rival males paired with unparasitized males

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

trials (8 of 18), while unparasitized males copulated with females in 27% of trials (5 of 18), a nonsignificant difference (unadjusted G statistic = 1.091, n = 36, p = .2979). However, power is low (ca. 50%) for the negative results in this experiment.

There were significant correlations between both the contact frequency and contact duration with the rival male by the parasitized male and the number of mites originally on each parasitized male (Figs. 5.1, 5.2; Spearman rank correlation coefficient; for contact frequency: $r_s = .375$, n = 17, p = .017; for contact durations: $r_s = .329$, n = 17, p = .046).

Of the unparasitized beetles paired with parasitized males, 50% of females and 44% of males received mites, although these data include any contacts that may have occurred after the taping period ended and before the beetles were removed from the dishes (approximately 8 hours). Nevertheless, there was no bias towards either sex in the rate of parasitism by *C. labidomerae*.

Experiment 2: The effect of mites on male contacts with unparasitized solitary males

Contact frequencies and durations with rival males were significantly greater for parasitized test males than for unparasitized test males (for contact frequencies, U = 192, p = .0056, Fig. 5.3; for contact durations, U = 230, p = .0368, Fig. 5.4; Table 5.2). The first contacts initiated by parasitized test males were also slightly longer on average than those initiated by unparasitized test males, though this difference was not significant (Table 5.2).

As in the previous experiment, there was no evidence of avoidance of parasitized males by rival males: contact frequencies and durations of the rival males in the parasitized group were not significantly different from contact frequencies and durations of the rival males in the unparasitized group (Table 5.2).

In contrast to the previous experiment, there were no significant correlations between the number of mites on the parasitized males and contact frequencies ($r_s = -.2$, n = 30, p = .2816) or contact durations by parasitized males ($r_s = -.013$, n = 30, p = .9463).

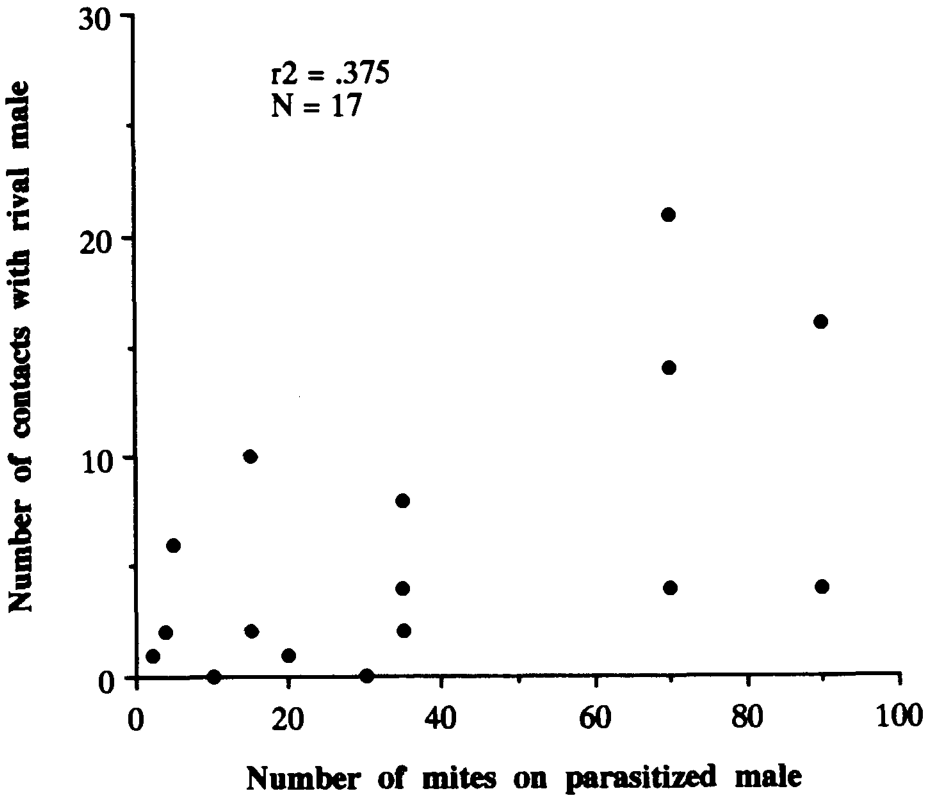

Figure 5.1 The relationship between the number of contacts initiated by parasitized males with unparasitized males over a six-hour period and the number of mites on the parasitized male ($p < 0.05$). One data point has been removed (see text).

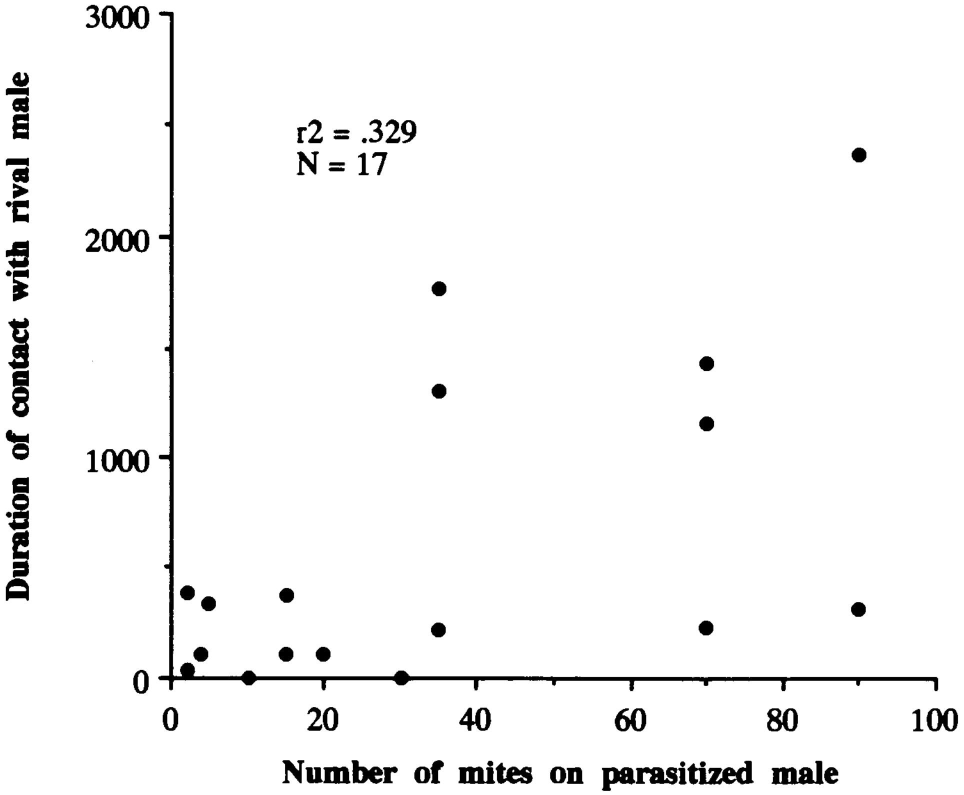

Figure 5.2 The relationship between the cumulative duration of contacts initiated with rival males by parasitized males over a six-hour period against the number of mites on the parasitized male ($p < 0.05$). One data point has been removed (see text).

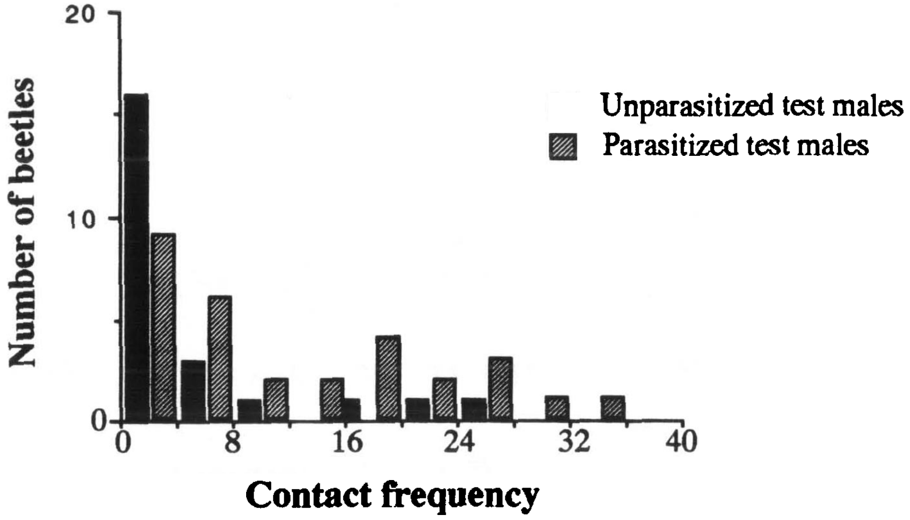

Figure 5.3 Contact frequencies between test males and unparasitized rival males over a two-hour period (dark bars - unparasitized; hatched bars - parasitized).

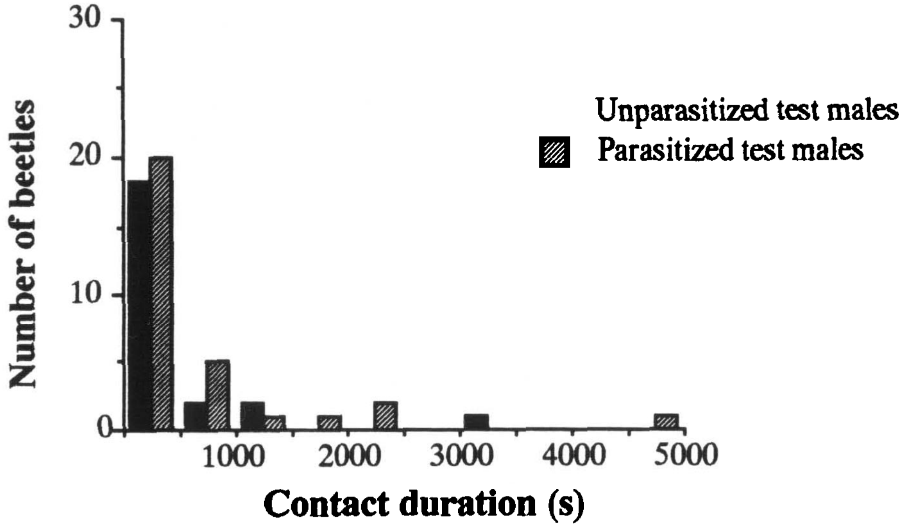

Figure 5.4 Cumulative contact durations between test males and unparasitized rival males over a two-hour period (dark bars - unparasitized; hatched bars - parasitized).

Table 5.2 Summary of frequencies and durations of contacts initiated by one male towards the other over two hours in an experiment designed to measure parasitized male contacts with unparasitized solitary males. Comparisons in the first and second rows (in bold type) are between the summed contact frequencies and durations of the parasitized and unparasitized groups. Subsequent comparisons are between parasitized and unparasitized test males (first and third rows - in bold type). Medians are in parentheses.

	Male type	N	Avg. # contacts initiated	Avg. contact duration/trial (s)	Avg. duration/first contact (s)
Parasitized group	Test male	30	**11 (8)***	**662 (398)***	**150 (71)**^{ns} (n = 26)
	Rival male[a]	30	6 (2)	225 (46)	--
Unparasitized group	Test male	23	**4 (1)**	**354 (11)**	**92 (56)** (n = 13)
	Rival male[b]	23	6 (1)	665 (14)	--

[a] denotes rival males paired with parasitized males
[b] denotes rival males paired with unparasitized males
* significant at $p < 0.05$, Mann Whitney U.

Experiment 3: The effect of copulation duration on mite transmission

There was a significant relationship between copulation duration and the number of mites transferred between parasitized males and unparasitized females (Kruskal-Wallis, H = 15.153, df = 2, p = .0005; Table 5.3). More mites transferred during the long copulation (900 s) than during either of the shorter copulations (nonparametric multiple comparisons, for 60 vs. 240, $p > 0.05$; for 60 vs. 900, Q = 3.55, $p < .001$; for 240 vs. 900, Q = 3.57, $p < .001$).

Table 5.3 Percentage of mites transferred from parasitized males to unparasitized females during copulations of three different durations: 60s, 240s, and 900s.

Copulation duration		**% mites transferred**		
	n	Mean	Median	Range
60 s	10	0.048	0.026	0 - 0.188
240 s	9	0.042	0.04	0 - 0.108
900 s	9	0.176	0.179	0.1 - 0.261

Discussion

These results demonstrate that parasitized beetles alter their behaviour in a way that increases contacts between beetles. In the laboratory, parasitism by *C. labidomerae* is associated with significantly elevated contact rates (Fig. 5.3) and, as a consequence, elevated contact durations (Fig. 5.4) between solitary parasitized and unparasitized males. These results substantiate similar trends detected in the experiment with copulating pairs in which parasitized males contacted unparasitized rivals slightly more often and for considerably longer on average.

Although there may be more, I discuss four of the most plausible explanations for the change in behaviour of parasitized males: 1) the change in behaviour represents adaptive modification of host behaviour by *C. labidomerae*; 2) the change in behaviour is a MLB adaptation to reduce mite burden by increasing the opportunities for mite transmission; 3) the change in behaviour is a MLB adaptation to increase reproductive output following a reduction in life expectancy; 4) the change is a non-adaptive, side-effect of parasitism, with no beneficial effects for either *C. labidomerae* or MLBs.

Is this change in male MLB contact rates an adaptive, parasite-induced modification of beetle behaviour? In Dobson's (1988) opinion, most known examples of host behavioural modification by direct life-cycle parasites "involve the release of a behavioural pattern that is performed in a novel context where otherwise it would be rarely performed in the absence of the pathogen. This primary stimulation to transmission is then complemented by secondary changes in the rates at which other, usually locomotory, activities are performed." The results presented here are consistent with Dobson's (1988) summary. To the extent that the contact rate between beetles influences mite transmission opportunities, the increased contact rate by parasitized male MLBs corresponds to a significant increase in transmission opportunities for *C. labidomerae.*

The primary difference in behaviour of parasitized and unparasitized males may have been in the level of activity in the petri dishes: parasitized males may have simply been more active than unparasitized males, perhaps as result of *C. labidomerae's* effect on their physiology. In the field, when males come within approximately 5-cm of each other, they contact each other more often than not (Dickinson, 1992). Thus, in these experiments, when parasitized males were in proximity to rivals, they may have been just as likely to contact rivals as were unparasitized males. By being more active, they increased the chances of encountering rival males, increasing per trial contact frequencies, and increasing the opportunities for parasite transmission. Because the cumulative contact durations per trial are not independent of contact frequencies, elevated activity of parasitized males could explain these results.

However, longer contacts may also be a parasite adaptation. Parasite-induced longer contact durations have been described in other host/parasite associations. For example, the median time to locate blood vessels for mosquitoes (*Aedes aegypti*) infected with *Plasmodium gallinaceum* is three times longer than that of uninfected mosquitoes (Rossignol et al., 1984). This change in mosquito behaviour may be induced by *P. gallinaceum* because longer probing times enhance transmission (Ribeiro et al., 1985). Similar relationships have been described in tse-tse flies infected with trypanosomes, in the *Trypanosoma rangeli*-infected blood-sucking bug *Rhodnius prolixus*, and in phlebotomine flies infected with *Leishmania* spp. parasites (Ribeiro et al., 1985). *C. labidomerae* may be inducing a similar change in MLB behaviour. Proportionally more mites move from an infected male to an uninfected female during longer copulation durations (Table 5.3). Because the results presented here provide evidence of longer contact durations (first contacts and cumulative contact durations by parasitized male MLBs; Tables 5.1, 5.2), induced changes in male behaviour may benefit *C. labidomerae* by enhancing transmission from parasitized hosts. The longer first contacts with rivals by parasitized males is particularly convincing evidence of an induced change in male behaviour: if, in the field, encounters between males reduce the probability of future encounters with the same beetle, then first contacts may be the primary opportunity for mite transmission. Longer first contacts would then enhance transmission.

Thus, adaptive behavioural modification of MLB behaviour by *C. labidomerae* is a plausible, consistent explanation of these results. Increased activity and/or aggression by parasitized male MLBs increases the encounter rate between beetles in the laboratory - an effect that, by definition, increases transmission opportunities. Once in contact, parasitized males may remain in contact longer, providing more opportunity for mites to migrate from the parasitized host. It is worth noting as well that these experiments document an actual increase in contact rate between hosts due to parasitism. In contrast, most studies describing behavioural changes in parasitized hosts, and ascribing these changes to parasite adaptations, speculate that the altered behaviour would increase contact rates between hosts without actually demonstrating this effect (Moore & Gotelli, 1990).

However, many symptoms associated with parasitism have no function, and are thought of as merely pathological consequences of infection which benefit neither parasite nor host (Read, 1990; Moore & Gotelli, 1990; Ewald, 1994). In fact, this "side effect" explanation serves as a null hypothesis against which to test for adaptive functions of the symptoms (Read, 1990; Ewald, 1994). A typical pathological side-effect of parasitism is debilitation or a decrease in general vigor of the host (Read, 1990; Moore & Gotelli, 1990; Ewald, 1994).

If the changes in parasitized MLB behaviour are merely pathological side-effects, would this explain how *C. labidomerae* consistently appears to increase activity or vigor of male MLBs? By feeding on haemolymph, thus draining the host's energy reserves, *C. labidomerae* should decrease, not increase, the contact frequencies or durations of parasitized MLBs. Ultimately, the side-effect explanation does not satisfactorily account for these results.

A more plausible alternative explanation for changes in parasitized male behaviour is that increased activity or aggressiveness by males are adaptive host responses to parasitism. Such host adaptations are well-documented, and normally function either as a defence against parasitism or to increase reproductive output in the face of a reduction in life expectancy or fecundity. Diarrhea, a familiar symptom of human parasitic infection, has been interpreted as an adaptive host response because it minimizes the duration of some intestinal infections (Ewald, 1994).

Could increased contact rates or durations be interpreted as a form of immune response by MLBs? If, by contacting other beetles, parasitized male MLBs were more likely to decrease than to increase parasite burden, it is conceivable that parasitized males exhibit an elevated tendency to contact other males in order to reduce mite burden. However, it is unclear how this mechanism could function. By contacting conspecifics more often, males may indeed transmit mites to other beetles, but may also receive mites from other beetles, which is especially likely in some populations of MLB in which mite incidence exceeds 90% (Chapter 4). If the contacted beetle has more mites, and transmission rates are proportional to mite burden, then the attacking beetle may receive more mites than he looses. Males must then accurately estimate their own parasite burden, and the parasite burden of those in their proximity, but this would be an extraordinary feat for any organism to do. Perhaps males with relatively more mites would reduce parasite burden on average by contacting other beetles, but if parasitized MLBs were to go to such lengths to reduce parasite burdens, then why not avoid parasitized conspecifics? There is no evidence that MLBs avoid parasitized mates (Chapter 4).

It is not necessarily the case that males would be better off with fewer mites. By attempting to lower parasite burden, males would run the risk of increasing the genetic variability of the mites they host by contracting mites from other beetles. Group selection between parasite colonies characterized by high within-group relatedness may lead to a reduction in parasite virulence; genetic-mixing may increase virulence (Ewald, 1994). Because *C. labidomerae* is arrhenotokous (Baker & Eickwort, 1975), colonies can be formed on hosts by a few, even single foundresses, producing highly-related groups of mites on different beetles. Acquiring unrelated mites may be risky if the hypothesized relationship between virulence and genetic variability holds for *C. labidomerae*.

It is unlikely that the change in MLB behaviour represents a host adaptation to reduce parasite burden. A more plausible alternative explanation for these results is that the change in parasitized male MLB behaviour represents an increase in reproductive effort in the face of reduced life expectancy (Minchella, 1985; Dobson, 1988). Such adaptive responses by hosts are not uncommon (see Minchella, 1985). Symptoms of parasitic infection may reflect adaptive compensations for parasite-induced decreases in life expentancy or fecundity (Mangel & Clark, 1988; see Roitberg et al., 1993).

Although *C. labidomerae* does not appear to have a severe effect on MLB longevity under normal laboratory conditions (Eickwort & Eickwort, 1986; Chapter 3), parasitized males are less likely to survive a period of starvation than unparasitized males (Chapter 3). If increasing activity and/or aggression leads to an increased probability of encountering and monopolizing a female, the change in parasitized male behaviour could be viewed as an adaptive compensation for reduced life expectancy under severe conditions (although, all beetles in the experiments described here were fed *ad libidum*). Several lines of evidence support this interpretation. If parasitized males were sensitive to a reduced life expectancy, they should invest relatively more in each encounter with copulating pairs, and on average, should be more successful in displacing rivals than unparasitized males, who would presumably value take-overs less as a mate-acquiring strategy. The 67% take-over success rate of parasitized males compared to the 33% success rate of unparasitized males supports the interpretation that males compensate for being parasitized. Moreover, if the probability of parasite-induced mortality increases with parasite burden (e.g. Lanciani, 1975; Chapter 3), then males with relatively more mites should more actively or more aggressively search for mates. The positive relationships between contact frequency and duration and parasite burden in the experiment with copulating pairs support this prediction (Figs. 5.1, 5.2). Finally, if male-male aggression in the absence of females functions to toss potential rivals from plants (Dickinson, 1992), then parasitized males not only may be more likely to encounter other males because they are more actively searching for females, but also may be more aggressive in these interactions because of the increased value of copulations that accompanies reduced life expectancy (Mangel & Clark, 1988).

Thus, these experiments offer some evidence in support of the alternative explanation that the change in male MLB behaviour is a host adaptation. However, if parasitized males were more actively searching for females because of a reduced life expectancy, we might reasonably expect a decrease in the time spent engaged with other males. It is surprising that the reverse appears to be true: parasitized males actually increase the time in contact with rivals (Table 5.1; 5.2). Often this contact involved "courtship", in which the parasitized male mounted and passively rode on the back of the other male. Although this is a characteristic behaviour of male MLBs in the lab (Baker & Eickwort, 1975), it is interesting that parasitized males increased rather than decreased the magnitude of this behaviour. By spending more time in contact with other males, parasitized males presumably have less time available to search for females. Thus, although displacing rivals from females may offer immediate fitness rewards to parasitized males, elevated contact frequencies and durations with solitary males is somewhat paradoxical under the explanation that parasitized males are more actively searching for females because of reduced life expectancy. By contrast, a parasite-induced elevation in activity easily explains the behaviour of males with and without females.

Although the host adaptation explanation is not entirely satisfactory, and a parasite adaptation modifying MLB behaviour stands as a completely consistent and parsimonious explanation for the full results presented here, these experiments cannot conclusively discriminate between these alternative explanations. As with other host/parasite associations, it may well be that the observed symptoms of parasitism by *C. labidomerae* are products of both host and parasite adaptations, and thus attempts to discriminate between the two are misguided (Ewald, 1994). If male MLBs were compensating for being parasitized by more actively searching for females, *C. labidomerae* would gain substantial benefits if this behaviour increased the chances of host contact. Thus, any elevation in activity level could benefit both host and parasite if the behaviour enhances transmission opportunities as well as the probability of encountering females.

If *C. labidomerae* is modifying MLB behaviour, what is the mechanism? As with any hypothesis about parasites and altered host behaviour, identification of specialized parasite traits for modifying host behaviour stands as the most conclusive proof of a parasite adaptation (Read, 1990). Until *C. labidomerae* or other podapolipids are examined for such traits, any hypothesized hormonal interaction between *C. labidomerae* and MLBs is mere speculation, although the feeding morphology of podapolipid mites may be an ideal delivery system for hormones (e.g. ecdysteroids) or other substances into beetle haemolymph (Lawrence, 1986). As with most behavioural modifications by direct life-cycle parasites, the proposed change in MLB behaviour induced by *C. labidomerae* is a relatively simple, generalized activity or aggression response which effectively increases the opportunities for transmission (Dobson, 1988), a far more simple change in behaviour than some of the more complex behavioural changes associated with indirect life-cycle parasites (Dobson, 1988; Moore & Gotelli, 1990).

Regardless of ultimate causation, perhaps the most interesting conclusion from these data is that a socially or sexually-transmitted parasite alters the sexual behaviour of its host, and that the change in behaviour is in a direction which demands a functional investigation, and most likely, a functional explanation. This is a novel result for a sexually-transmitted parasite (see, by comparison, Møller, 1993a). The focus of the vast majority of research on social or sexual behaviour and parasites has been on host adaptations (e.g. mate choice) in response to selection from parasites (see for example Read, 1990 or Moore & Gotelli, 1990). Although the relationship between social evolution and parasites may indeed be cryptic (Freeland, 1976), and difficult to interpret (Read, 1990), these results suggest that the role of sexually-transmitted parasites in shaping the outcome of social or sexual interactions of their hosts has been significantly underestimated. Considering the effect of host sexual or social behaviour on the evolution of parasite traits, rather than solely vice versa, may broaden our understanding of the epidemiology and behaviour of directly-transmitted parasites and, interestingly, broaden our understanding of the sexual and social behaviour of their hosts as well.

Chapter 6

General Discussion and Conclusion

Broadly considered, the transmission success of parasites depends upon two aspects of host behaviour: on the behaviour of parasitized hosts themselves and on the behaviour of other hosts towards parasitized individuals. Describing the former is one goal of a typical study of parasite manipulation of host behaviour, and describing the latter is a goal of studies attempting to explain mate choice as a means of parasite avoidance. These two areas of research seek to understand two different, but related, aspects of host/parasite evolution, with the shared aim of attempting to understand the effect of host behaviour on parasite transmission (see Sheldon, 1993). In this thesis I applied to a single host/parasite system - the milkweed leaf beetle parasitized by *Chrysomelobia labidomerae* - these dual approaches to the interactions between a sexually-transmitted parasite and its host.

I asked two straightforward questions: 1) Do unparasitized beetles avoid parasitized beetles more than expected - potentially an adaptation to minimize the risk of acquiring a potentially costly parasite, and 2) Do parasitized beetles contact other beetles more than expected - potentially as a result of a parasite adaptation for increasing transmission success?

In a preliminary experiment, described in Chapter 3, I showed that *C. labidomerae* can reduce the longevity of milkweed leaf beetles (MLBs) under nutritional stress. Although acquiring *C. labidomerae* may be a cost of mating with a parasitized individual, I found no evidence to support the hypothesis that MLBs avoid parasitized mates (Chapter 4). The maintenance of random mating under selection from potentially pathogenic parasites is somewhat perplexing, but may reflect even more substantial selection against non-random mating in the form of costs associated with searching for unparasitized mates. Random mating may persist in host species under selection from sexually-transmitted parasites when the ecological and demographic characteristics of the host population make searching for mates costly - for example, in a highly dispersed, short-lived host species with significant risks of mortality from biotic or abiotic sources. A study comparing several beetle species parasitized by *C. labidomerae* may reveal that parasite avoidance more often occurs in species with relatively small search costs - that is, in aggregative, longer-lived species with relatively small risks of mortality associated with searching for mates.

C. labidomerae does have a direct effect on MLB behaviour, one that corresponds to an *a priori* prediction about how a sexually-transmitted parasite should modify host behaviour to increase transmission opportunities. Parasitized MLB males display an elevated tendency to contact unparasitized males, and are more likely to displace other males from females (Chapter 5). Simply by increasing the frequency, and in some cases the duration of contacts with conspecifics, the behaviour of parasitized males increases the transmission opportunities for *C. labidomerae*. On the strength of the close correspondence between the predicted and the observed effect of *C. labidomerae* on MLB behaviour, I argue that the behavioural change in male MLBs may be induced by *C. labidomerae*, and may be evidence of a parasite adaptation for increasing transmission success.

Unfortunately, the latter conclusion must remain tentative until more evidence is available to determine the significance of the altered behaviour of parasitized MLBs. Follow-up experiments are needed in order to determine the beneficiary (if there is one) of these behavioural changes - *C. labidomerae*, MLBs, or both. Although discriminating between alternative explanations for the observed effects of parasites on their hosts is often difficult, as many authors have recently emphasized (e.g. Moore & Gotelli, 1990; Read, 1990; Keymer & Read, 1991), there are several promising experimental and observational approaches that could properly identify who benefits from the behavioural change by parasitized MLBs.

As noted in Chapter 5, in order for parasites to alter host behaviour adaptively, they must possess specialized morphological or physiological traits for that purpose. A stronger case could be made for the altered MLB behaviour being a *C. labidomerae* adaptation if specialized structures were identified in *C. labidomerae* that appear to function as MLB physiology or behaviour modifiers.

A more ecological approach would characterize the function of the change in MLB behaviour. Comparative studies can be useful tools in characterizing the current function of parasite-induced behavioural changes (Moore & Gotelli, 1990; McLennan & Brooks, 1991). For example, *Chrysomelobia* spp. parasitize 7 species of chrysomelid beetles. It would be useful to compare other *Chrysomelobia* hosts for behavioural changes similar to those observed by parasitized MLBs. If the hypothesized parasite trait for modifying host behaviour arose before the radiation and specialization of the genus *Chrysomelobia*, then beetle species with ecological and behavioural characteristics similar to MLBs might be expected to exhibit similar changes in behaviour when parasitized by *Chrysomelobia* (*sensu* Yan et al., 1994).

Another approach that could discriminate between various explanations for the effect of *C. labidomerae* on MLB behaviour would involve experimentally falsifying the two conjectures that *C. labidomerae* and/or MLBs accrue fitness benefits from the change in MLB behaviour under parasitism. For example, a convincing test of the hypothesis that parasitized MLBs are responding to the reduction in life expectancy could be made by demonstrating that following reductions in life expectancy not associated with parasitism by *C. labidomerae*, MLBs do not respond by increasing their tendency to contact other males. Life expectancy has been experimentally manipulated elsewhere (Roitberg et al., 1993), so there is promise in this approach. A useful part of this study would be the characterization of the the function of male-male interactions in the absence of both parasites and female MLBs. By understanding the function of male-male interactions, the significance of changes in parasitized male behaviour could then be better understood.

Likewise, because the hypothesis that *C. labidomerae* benefits from the change in MLB behaviour rests on the assumption that the change increases the transmission opportunities for the mites, falsifying this hypothesis requires measuring the transmission rates (and subsequently the survival and fecundity rates) of *C. labidomerae* under different rates of contact between MLBs. If *C. labidomerae* does not appear to benefit by increasing its rate of spread through a beetle population when contact rates are experimentally manipulated, then the parasite adaptation hypothesis for the changes in MLB behaviour would be falsified.

Whatever the ultimate causes of the change in MLB behaviour (some of which were discussed in Chapter 5), there can be little doubt that parasitized MLBs do not behave like unparasitized MLBs, and this behavioural change can affect both the frequency and the outcome of social or sexual encounters between beetles. Social or sexual behaviour of animals may thus be influenced by parasites in a manner not acknowledged in current reviews of the effect of parasites on the social behaviour of their hosts (e.g., Keymer & Read, 1991). If sexually-transmitted and other directly-transmitted parasites like *C. labidomerae* do modify host behaviour, then the role of parasites in shaping host social or sexual behaviour becomes not only a question of how parasites shape host characteristics like group size or mate choice, for example - characteristics which can be usefully thought of as defenses against parasites - but also a question of what characteristics of host social or sexual behaviour are at least partially dependent on the direct physiological interaction between host and parasite.

Given the recognition that parasites promote the evolution of host social characteristics which function to reduce the transmission rate of parasites between hosts (Keymer & Read, 1991), behavioural ecologists should readily accept the corollary argument proposed and empirically-supported in this thesis: that sexually-transmitted parasites can increase transmission opportunities by modifying the social or sexual behaviour of their hosts. I strongly recommend that where host social or sexual characteristics are identified which function to reduce the chances of parasite transmission, researchers look for concomitant parasite characteristics which function to increase the success with which parasites move from one host to another.

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