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**ECTOPARASITISM OF ODONATE HOSTS :
HOST RESPONSE TO PARASITISM AND HOST SEX BIAS IN
PARASITISM**

by

NANCY JACYNTHÉ LÉONARD

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements for the degree of
Master of Science

Department of Biology

Carleton University

Ottawa, Ontario

December 1998

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acceptance of the thesis

**ECTOPARASITISM OF ODONATE HOSTS:
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Submitted by
Nancy Leonard, B.Sc.

in partial fulfilment of the requirements
for the degree of Master of Science

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Carleton University
January 11, 1999

Abstract

In the first chapter, I found that natural high levels of *Limnochares americana* (Lundblad) tend to negatively affect the survivorship and significantly reduce the time to female maturation of its damselfly host, *Enallagma ebrium* (Hagen). I observed that damselflies increase their grooming behaviours when they are being colonised by mite larvae. However, I did not observe any adaptive host response, i.e., an increase in reproductive effort, by adult males or females.

In the second chapter, I investigated the causes of sex biases in parasitism by *L. americana* observed for field-caught *Nehalennia irene* (Hagen) and *Leucorrhinia frigida* (Selys). I also used *Enallagma ebrium* (Hagen), to test for the occurrence of a sex bias in engorgement size of *L. americana*. I found that *N. irene* showed no sex biases in parasitism in the laboratory whereas *L. frigida* females were more susceptible to mite colonisation than males. I also found that mites attained a larger size on *E. ebrium* females in the laboratory but not in the field.

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Chapter 1

Effects of parasites on life history traits and behaviour of their hosts: a study using *Enallagma ebrium* (Hagen) (Odonata: Coenagrionidae) and *Limnochares americana* (Lundblad) (Hydrachnidia: Limnocharidae).

Introduction

Numerous examples exist in which host fitness is reduced by parasites; parasitized hosts can show reduced survivorship (Lanciani 1979, 1982, 1986, Robinson 1983), reduced fecundity (Lanciani 1975), reduced mating success (Forbes 1991a, b), reduced competitiveness in obtaining food (Anderson 1978, Mohan and Rao 1984), and reduced ability in escaping predators (Anderson 1978, LaMuyon and Eisner 1990). If parasite prevalence is high, then parasites can negatively impact host populations (May and Anderson 1993, Jaenike 1996). These negative impacts can affect interspecific competition (e.g., chironomid midges parasitized by mermithid nematodes: LeSage and Harrison 1980) and influence distributions of species (e.g., water boatmen parasitized by *Eylais* mites: Scudder 1983, cf. Smith 1988) thereby affecting community structure (Price 1980, Minchella and Scott 1991, Toft 1991).

Traditionally, parasites were thought to have an evolutionary advantage over their hosts because their shorter generation times should allow them to escape host defences and exploit hosts to their own end (Minchella 1985). However, many ecologists now view both host and parasite as influencing the degree to which hosts show reductions in fitness once parasitized (Minchella 1985, Minchella et al. 1985, Keymer and Read 1991, Poulin 1994). Some hosts are believed to be able to influence the parasite's impact on host fitness by controlling allocation of host resources, or by the hosts changing their behaviour in ways that lessen parasite impact (Minchella 1985).

Many examples exist where, as a first line of defence, hosts change their behaviour to control parasites or to lessen the effects of parasitism. For example, hosts avoid areas infested with ectoparasites (e.g., cattle avoiding areas with high numbers of ticks: Hart 1990). Hosts also may increase the amount of time spent grooming to decrease the number of ectoparasites that successfully colonise them (e.g., damselflies grooming off ectoparasites: Forbes and Baker 1990, Baker and Smith 1997). Importantly, to be able to respond to parasites adaptively, hosts must be able to detect attack by parasites, or instances in which they are parasitized. Elicitation of anti-parasite behaviour (e.g., grooming) is indicative of parasite detection by hosts.

Research on the manifold ways hosts respond to parasites is important because of the growing interest in biological control of pests (Hoy et al. 1983, Smith 1983) and the possible influence of host response on the effectiveness of the parasite chosen for biocontrol. Yet, there have been few studies investigating how invertebrates reduce the costs of parasitism (e.g., Forbes and Baker 1990), as compared to the many studies that attribute reductions in reproduction or survival of invertebrate hosts to their parasites (Lanciani 1975, Smith and McIver 1984, Smith 1989). Additionally, we have only a basic understanding of the invertebrate immune response in controlling both endoparasites and ectoparasites (Lackie 1980; Brehélin 1986, Gotz 1986, Ratcliffe 1993). One common immune response by invertebrates is melanotic encapsulation of foreign objects, including feeding tubes of ectoparasitic mites (Lackie 1980, Åbro 1982, Wakelin 1984). Other responses are less obvious and involve changes in allocation of energy or time to certain life history traits or behaviours.

Hosts may change their allocation of resources to life history traits or behaviours as a defence against parasites because such responses may be less costly than many physiological immune responses (Forbes 1993). For example, the parasitized hosts may increase the amount of time spent foraging to offset the energy lost to the parasite (Reilly and McCarthy 1991). Another type of response that is less obvious may involve the host rerouting the energy that would otherwise go into physiological defences

into reproduction. For example, hosts may be expected to increase their investment in current reproduction (e.g., egg laying rates) following initial colonisation by parasites that reduce probability of future survival (otherwise, hosts may die before they realise a greater return on investment in reproduction, Forbes 1993). Such changes in host resource allocation have been reported for snails that show an increase in egg production once parasitized by trematode larvae (Minchella and Loverde 1981). The authors observed this host response before the larval trematodes had become even more costly to its host by multiplying or growing substantially. In such cases involving differential allocation of host resources, the parasitized host is expected to have lower fitness than unparasitized hosts. However, the parasitized host may realise a higher fitness than if it had not reallocated its resources.

In this chapter, I examine responses by the damselfly host *Enallagma ebrium* (Hagen) to experimental infestation by the mite, *Limnochares americana* (Lundblad). To this end, I first assessed natural levels of parasitism for pre-reproductive and adult male and female damselflies ca. every 2 weeks during their flight season. I did this first step to determine levels of parasitism to use in my experiments. I then examined whether damselflies responded to attack by mites by increasing their grooming rates, which also would indicate that they were able to detect mites, and possibly perceive mites as 'costly'. Following this, I examined the extent to

which natural levels of mite parasitism reduced survivorship of damselflies of different ages or sexes, or delayed maturation of females, placed under conditions of partial or complete food deprivation. Providing that damselflies were able to detect mite attack and mites were associated with reductions in survivorship (or delays in maturation), then damselflies should respond to mite parasitism by altering investment in current reproduction, such as mate-searching activity. I tested this prediction using adult male damselflies, but also explored any changes in behaviour of pre-reproductive and mature males and females, associated with experimental parasitism.

Methods

Study species

I chose to study *E. ebrium* because it is an abundant, medium-sized damselfly (26-33 mm) that is relatively easy to locate (Walker 1953). This species also has a relatively long flight season (ca. 2 months in Eastern Ontario; Walker 1953). Additionally, basic information on its behaviour, reproduction, and life history is known, particularly in relation to parasitism by *Arrenurus* mites (Forbes 1991a, Forbes and Baker 1991). Lastly, methods for its collection, housing, and transport are well developed.

Limnochares americana mite larvae are suitable for study because they are easily collected and relatively large compared to *E. ebrium* (ca. 2 % of its length based on the average length of field engorged mites, 0.57 mm: N.J. Léonard unpubl. data). Using *L. americana*, I was able to control the timing and approximate numbers of mite larvae colonising damselflies. Finally, *L. americana* is a generalist parasite on odonates (Smith and Oliver 1986); thus, comparisons are possible among host species with respect to variation in susceptibility to, and engorgement by, *L. americana* (e.g., Chapter 2).

The life cycle of parasitic water mites, including *L. americana*, are detailed by Smith (1988). Below, I briefly review some relevant features of its life cycle. Adult female mites lay ca. 700 eggs in clutches on submerged stems of emergent vegetation (Smith and Oliver 1986). Upon hatching, the mite larvae swim to the water surface and crawl up on emergent vegetation. When an adult odonate returns to the pond and perches on the emergent vegetation, these larval mites climb on to the venter of the odonate and attach to it by inserting their chelicerae through the host's cuticle. Shortly thereafter, the larvae begin to engorge. Once fully engorged, the larval mite drops off the host (when the host is over water), and completes its life cycle as an aquatic predator (Figure 1).

Figure 1.1: Simplified life cycle of *L. americana* (Lundblad) modified from Efford (1963), Smith and Oliver (1986), and Smith and Cook (1991). 1) Adult females lay eggs on aquatic stems and larval mites hatch. 2) Larval mites climb onto emergent vegetation and attach on to an adult damselfly. 3) Larval mites engorged on the venter of an adult damselfly. 4) Engorged mites detach and return to the water. 5) The engorged mite matures and becomes an aquatic predator.

Surveys and collections of damselflies for use in experiments

I surveyed and collected damselflies from Two-Island Lake, which is a large freshwater marsh (ca. 50 ha) surrounded by fields and wooded areas. Two-Island Lake is less than 10 km west of the Queen's University Biological Station (QUBS), which is located less than 2 km west of Chaffey's Locks Ontario, Canada (44° 35' N; 76° 19' W). I surveyed *E. ebrium* in the field to determine natural levels of mite abundance, and to assess whether intensity of *L. americana* mites was related to sex or age of damselflies. Surveys were done on the 15-16 and 19 June 1997 (survey 1), 7-8 July 1997 (survey 2) and 21-23 and 27 July 1997 (survey 3). I captured individuals using an aerial net, and recorded the number of *L. americana* mites engorging on the individual and then marked the individual's wing with a permanent marker (Staedtler) to preclude resampling it, following its release (cf. Forbes 1991a).

All damselflies used in my experiments were field-caught individuals with only *Arrenurus* mite species engorging on them (e.g., *Arrenurus magnicaudatus*, Forbes and Baker 1991). By using damselflies that still retained their *Arrenurus* mites, I was able to control degree of current parasitism. If damselflies had lost their *Arrenurus* mites and only had mite scars, I could not control for past levels of parasitism because scars are difficult to count as damselflies age. I was certain also that all sexually mature damselflies used in experiments were about the same age, because

Arrenurus mites colonise damselflies only during host emergence and detach from the sexually mature damselfly host more or less simultaneously (cf. Mitchell 1968; Smith and Cook 1991). Due to the possible effects of these *Arrenurus* mites on the damselfly host (Forbes 1991a), I controlled for mite numbers by placing same-sex damselflies with same or similar numbers of *Arrenurus* mites into different treatments within an experiment. I transported all damselflies to the laboratory in portable cages made of a wooden frame and wire screen mesh (30 cm X 30 cm X 40 cm). Cages were supplied with water-soaked paper towels to prevent desiccation of damselflies during transport (Hinneking 1987).

Collections of mite eggs and rearing of mite larvae

I collected mite egg masses from a Sphagnum bog less than 15 km west of QUBS. I first transported stems of emergent vegetation with *L. americana* egg masses to the laboratory. Stems were embedded in floral green sponge, to simulate eggs laid on vertical stems, and then submerged in water-filled plastic containers (30 cm x 20 cm x 10 cm). I used a heat lamp to provide heat and light with which I could control crawling activity of the mite larvae. These mite larvae were used in grooming experiments, as well as in all other experiments in which individuals were assigned to an experimental 'parasitized' category. To challenge experimental subjects with parasites (e.g., grooming experiments) or to parasitize damselflies

(e.g., survivorship experiments), damselflies were held by their wings at the rim of the container that housed mite larvae, until some mites colonised the damselfly (see details below and Appendix 1 for numbers of colonising mites per experiment or per category of test subject). Controls were treated in the same way, but held slightly distant to the container so mites could not crawl on them.

Grooming in relation to colonisation by mites

I tested whether male and female damselflies of both age groups (teneral or pre-reproductive, and adult) increased the time they spent grooming relative to controls, after being colonised by low or high numbers of *L. americana* mites. In the experiments, field-caught damselflies were matched by sex and age (pre-reproductive adults called tenerals, or sexually mature adults). Matched damselflies were housed individually in numbered plastic cups (following Leung and Forbes 1997 and detailed below) before being randomly assigned to either the control or mite treatment (low or high numbers of *L. americana*).

In total, 6 experiments were done. For the first 4 experiments involving teneral males and females (40 and 46, respectively), and adult males and females (40 and 40), damselflies assigned to the low parasitism treatment were challenged with 4-6 colonising mites. In this experiment as in other experiments, I could not control the exact number of mites

colonising damselflies without excessive interference (e.g., continually knocking mites off damselflies). The first damselfly from a matched pair received its treatment and was then returned to its cup, where it was left for 1 minute before being observed for 5 minutes. During those 5 minutes, I recorded and timed all grooming behaviours observed (Table 1.1). Matched pairs were observed within 10 minutes of one another (order of observation alternated between control and parasitized individuals).

For the next 2 experiments involving adult males and females (40 and 42), controls were treated as above, whereas damselflies assigned to the high parasitism treatment were challenged with 10-15 mites. Here again, order of observation of controls and other test subjects was alternated, but grooming behaviours were timed immediately after the damselfly was returned to its cup (for a total of 6 minutes). This change in protocol allowed me to record any grooming that may have occurred in the first minute following the administration of the treatment. In the previous experiment, I noted that damselflies engaged vigorously in grooming behaviours immediately following colonisation by mites.

Survivorship experiments

I conducted two types of survivorship experiments, using males and females from both age cohorts. In the first type of experiment, I deprived damselflies of food immediately after they received their treatment

(complete food deprivation). In the second type of experiment, I fed damselflies for 3 days in outdoor insectaries following treatment to increase the likelihood that mites would start engorging (because mites may delay engorgement if hosts are initially deprived of food, Lanciani 1976) and then food deprived hosts for the remainder of the experiment (partial food deprivation). In this second type of experiment, I also examined the effect of low and high numbers of *L. americana* larvae on damselfly survivorship.

For experiments based on complete food deprivation, I assigned the damselflies, either males or the females that belonged to an age group (either teneral or adult) to the control or low parasitism treatment (approximately 2 mites; see Appendix 1 for details). After administering the treatments (parasitism or control) I placed damselflies individually into numbered plastic cups within a cool and dim room to simulate naturally-occurring conditions of cool, wet weather and periods of low activity and reduced feeding (mean temperature =16.9°C; range 14.0-19.5°C; cf. Forbes and Baker 1991). Following Leung and Forbes (1997), each numbered cup (275 ml) held one damselfly and was provided with a perforated aluminium foil lid, a 5-cm applicator stick (Smith & Nephew Inc.) that served as a perch and ca. 10 ml of water to prevent desiccation of damselflies. The cups also had an aluminium screen "false bottom" that was skewered on the applicator stick and which prevented the damselfly from becoming trapped in the water's surface tension. I checked individuals

every 12 h and recorded the number of half days they survived, and the number of engorging *Arrenurus* (see Appendix 3) and *L. americana* mites.

At the end of this and most other experiments, I measured forewing lengths of each damselfly, as an index of body size (Forbes and Baker 1991). The wing was measured (to the nearest 0.01 mm) from its nodus to its end, using either a pocket comparator (Spi Scale Lupe 10X Spi model # 40-146-3) or digital solar callipers (Mitutoyo). Body size has been associated with survival and with fecundity of *E. ebrium* damselflies (Forbes and Baker 1991) and therefore it should be controlled in experiments assessing impacts of parasites on fitness traits.

For experiments based on partial food deprivation, I first collected sufficient numbers of damselflies (see below) of the same age and same sex. In these experiments, damselflies with either experimentally low (approximately 2 mites; Appendix 1) or high (approximately 4 mites; Appendix 1) numbers of *L. americana* mites were compared to controls. I treated damselflies as above, colour marked their wings with respect to treatment category (cf. Forbes 1991a) and then released them into outdoor insectaries where they were allowed to forage for 3 days. Wing marking was necessary because *L. americana* mites may be completely lost from some parasitized individuals, or control individuals may become accidentally parasitized (to reduce this possibility, experimentally parasitized and control damselflies were transported to insectaries in different cages). If

Table 1.1: Description of the grooming behaviours of *E. ebrium* modified from Baker and Smith (1997).

behaviour	description
leg grooming	damselfly rubs two legs together. For example, both prothoracic legs are rubbed together, or a prothoracic leg is rubbed against a mesathoracic leg or a mesathoracic leg is rubbed against a metathoracic leg.
eye grooming	damselfly rubs one or both prothoracic leg(s) over one or both eye(s) followed by rubbing both prothoracic legs together and/or passing the prothoracic leg through the mandibles

either of these 2 scenarios occurred (< 1% of cases), then the damselfly was not included in subsequent analyses.

E. ebrium engage in 'normal' behaviour when placed in an insectarium (Forbes and Leung 1995). The insectarium was constructed of aluminium poles with mesh walls on all four sides (3.7 m x 3.7 m x 2.0 m, described by Forbes and Leung 1995) with a plastic wading pool (1.5 m x 20 cm) containing water to prevent desiccation, which is a major source of mortality in laboratory or semi-natural conditions (Hinneking 1987). Controls and parasitized individuals in the same experiment were kept within the same insectarium to ensure that they were exposed to similar conditions. I released ca. 3,000 field-collected chironomid midges per insectarium per day as food for damselflies. I also inspected the insectarium each day and removed any predators such as spiders, other insects (dragonflies or robber flies), and frogs, that had managed to enter the insectarium. All damselflies used in these experiments (except for adult females) were captured, treated and placed in an insectarium in a single day. Adult females were kept overnight in plastic cups to allow them to oviposit any eggs prior to being treated. I found in preliminary experiments that adult females often drowned in the wading pools partially due to the absence of vegetation on which to crawl/perch during ovipositing.

After 3 days of feeding, all surviving damselflies that were recaptured (some died and/or were lost) were placed in numbered plastic cups in a cool and dim room and deprived of food, as outlined above. I recorded survivorship, number of engorging *Arrenurus* (see Appendix 4) and *L. americana* mites and measured forewings, as described above. Protocols were the same for the experiments comparing damselflies with low or high numbers of *L. americana* mites to controls. Additionally, after being recaptured, all damselflies were placed individually in plastic cups housed in a dim room whose mean temperature (measured twice daily) was 18.7°C for the duration of the experiment (range: 15.0-22.0°C).

Maturation of females

As an additional test for the 'cost' of being parasitism by *L. americana* mites, I tested whether parasitism by high numbers (approximately 4 mites, see Appendix 1) of *L. americana* mites delayed maturation of teneral female damselflies. I collected teneral females and assigned them to either the control or parasitized treatment. Females received their treatment on the day they were caught. Females were released later that same day into an insectarium where they were allowed to forage for 11 days on field-collected chironomids, as described above. Day 11 was chosen because some females showed colour change on the 10th day, indicating they had attained sexual maturity (Walker 1953). I collected surviving females (4

controls and 4 parasitized), counted their number of engorging *Arrenurus* (see Appendix 5) and *L. americana* mites, and measured their forewings. These females were placed individually in microcentrifuge tubes filled with 95% ethanol. I later dissected them and counted their mature eggs (following Forbes and Baker 1991). Importantly, control females had significantly more *Arrenurus* mites (median=8.0, interquartile range, IQR, =8.0-9.0) than females that received *L. americana* mites (median =3.5, IQR=3.0-4.5). As such, inferences from results must be made with caution, but the lower number of *Arrenurus* mites on the damselflies parasitized by *L. americana* mites probably did not play an important role in decreasing the number of eggs found in the abdomen of these damselflies.

Behavioural responses of adult males

I examined whether adult males increased their reproductive effort (increase in mate searching, misdirected sexual behaviour or trial at which successful tandem occurred) or changed the proportion of time spent engaged in other non-reproductive behaviours (flight, foraging flight or inspection) in response to experimental parasitism by *L. americana* mites. To this end, I performed three experiments, where control males were compared to experimental males, which differed in their numbers of, and degree of engorgement by, *L. americana* mites. For the first experiment, I collected adult male damselflies, and randomly assigned the damselflies to

either the control or parasitized treatments with low numbers of *L. americana* mites (approximately 2, see Appendix 1). Once all damselflies received their treatment, they were transported to the insectarium and released. I recorded behavioural responses of males (described in Table 1.2) to male and female models on the following day from 10:00-12:30 h. As such, mites were only beginning to engorge on experimental males. I did not feed damselflies during this experiment.

The models used were adult males and females recently killed by thoracic compression (Forbes 1991a); this method does not result in external damage. Models were mounted on a grass stem using an insect pin (Forbes 1991a) that passed through the damselfly's thorax and the last abdominal segment. The head of the pin was embedded into the dorsal portion of the thorax to render it invisible. Presentation of models to damselflies and behavioural records were made as described by Forbes (1991b). Basically, I first selected a perched damselfly (focal damselfly) and presented it with the adult male model by pushing it "side on" toward the perched damselfly. I recorded whether the focal damselfly remained perched. If it flew, I recorded all behaviours and their duration until the focal damselfly perched again. The male model was then presented 2 more times and behaviours recorded as above. Following this, the female model was presented to the focal damselfly (only tested the response of adult males) up to a maximum of 3 times or until the focal adult male took the

female model in tandem. The trial ended if tandem occurred because the adult male had to be physically removed from the female. I scored whether the focal adult male was successful in forming tandem with the female model on presentation 1, 2, 3 or not at all (score of 4).

Observations of control and experimental adult males were alternated to reduce the influence of a change in microclimatic conditions on male behaviour. After each damselfly was tested, he was placed into a numbered plastic cup. I measured his forewing length and the number of engorging *Arrenurus* and *L. americana* mites. In total, I had a sample size of 21 pairs of males.

In the next two experiments, I compared control adult males to adult males with either low (approximately 2 mites) or high numbers (approximately 3 mites) of partially-engorged *L. americana* mites (see Appendix 1). In the experiment where parasitized males had low numbers of partially-engorged mites, I used 29 pairs of teneral males and allowed them to mature before performing the model presentations. I scored males as sexually mature once they attained adult coloration (Walker 1953) 4-5 days following their release into the insectarium, which was supplied with field-collected midges daily. In the experiment where parasitized males had high numbers of partially-engorged mites, 14 pairs of adult males were used. In this experiment, males also were released inside the insectaries where they could forage, after they received their treatments.

Approximately 4 days later, and once the mites had partially engorged, I scored behavioural responses of males to the male and female models (teneral: from 10:30-12:00 h; adults: from 13:00-16:00 h). I then captured males, recorded their numbers of engorging *Arrenurus* and *L. americana* mites, and measured their forewing lengths.

Table 1.2: Description of *E. ebrium* behaviours, both reproductive and non-reproductive, recorded when presented with the male and/or female models.

behaviour	description
<i>non-reproductive</i>	
flight	the damselfly flies to a new perch without mate searching, inspecting the model, or foraging.
inspection	the damselfly hovers near the model while facing it or the damselfly flies toward the model and 'rams' it with his head once or repeatedly, and biting the model. The damselfly also may perch on the wings, eyes, or the main body of the model.
foraging flight	the damselfly flies either horizontally or vertically along the walls of the insectarium or along stems of vegetation, occasionally gleaning the surface of these stems or other substrate.
<i>reproductive*</i>	
mate searching	the damselfly flies without gleaning the walls or vegetation stems; and may inspect other male damselflies it encounters.
misdirected sexual behaviour	the damselfly attempts to form tandem with the male model or another male within the insectarium, by landing on the male or model and probing its pronotum with his claspers.
successful tandem	the damselfly lands on the thorax of the female model, grasps the model's pronotum with his claspers.

* Reproductive behaviours were recorded only for the response of adult male damselflies to the male and female damselfly models.

Behavioural responses of adult females and teneral

I also performed 3 experiments to look at the effects of high numbers of partially-engorged *L. americana* mites on the non-reproductive behaviours of adult female and teneral (male and female) damselflies when presented with the male model. Fifteen pairs of teneral males received their treatment on the same day of capture, whereas 17 pairs of teneral and 24 pairs of adult females were kept overnight in cups to allow for more mite larvae to hatch (tenerals) and also to allow adult females to lay eggs. After 3-4 days in the insectaries provided with food, I tested the behavioural responses of damselflies to adult male models (between 13:00- 16:00 h). The trials were performed as described above and behaviours described in Table 1.2 were recorded and timed.

Statistical methods

Most analyses were done using Statistica (Statsoft Inc. 1994); Chi-square tests were done using SYSTAT (Wilkinson 1991). I did parametric tests whenever possible, but when assumptions were violated, non-parametric tests were used. Details of statistical analyses are outlined below (see results). To correct for multiple tests, I used the sequential Bonferroni procedure, which is regarded as being highly conservative (Rice 1989).

Results

Survey of mite abundance on E. ebrium damselflies

I found that 29 % of all individuals sampled had *L. americana* mites; abundance ranged from 0 to 12 mites per individual (N = 406; Figure 1.2). Mean intensity (number of mites per parasitized host) was 2.3 ± 2.2 mites (N=119). Damselflies had a low number of *L. americana* mites at the beginning of their flight season (Survey 1), with mite numbers increasing for adult damselflies at the middle and end of the flight season (surveys 2 and 3), probably due to repeated exposure of individuals. I did not find a sex bias in mite intensity (Table 1.3: survey 1 excluded from analysis because of low sample size). However in examining the prevalence data (number of hosts with 1 or more *L. americana* mites/ number of hosts examined) and associated 95% confidence intervals (calculated following Snedecor and Cochran 1980), I found a sex bias in parasitism of adults, with females having a higher prevalence. Also, more adult males were parasitized by *L. americana* mites as compared to teneral males in surveys 2 and 3 (tenerals were not collected in survey 1), whereas adult females had a higher prevalence than tenerals only in the third survey (Table 1.4).

Figure 1.2: The number of *L. americana* mites attached on *E. ebrium* damselflies during survey 1 to 3. This graph includes all adults and teneral that were sampled in survey 1 to 3.

Figure 1.3: The mean intensity of *L. americana* mites found on *E. ebrium* damselflies in surveys 1 to 3. The empty bars represent the males and the hatched bars represent the females. The x-axis is labelled with the survey number separated by a dash from the age group, adult (A) or teneral (T). The error bars represent the standard deviation, and the sample size (N) per survey for males (M) and females (F) are shown.

Table 1.3: Effect of host sex on the median intensity of *L. americana* mites engorging on adult *E. cbrium* damselflies, for surveys 2 and 3. Given are the sample size (N), the median number of *L. americana* mites, the 25th and 75th interquartile range (IQR) and the Mann-Whitney U-test results.

survey	date	sex	N	median	IQR	U	p-value
2	7 July 1997	female	15	2.0	1.0-4.0	140.0	0.44
		male	22	2.0	1.0-4.0		
3	23 July 1997	female	34	1.5	1.0-2.5	364.5	0.49
		male	24	1.0	1.0-2.0		

Table 1.4: Prevalence of parasitism in *E. ebrium*. Shown are prevalence and 95% confidence intervals and sample size (N) for each sex and age group within surveys 1 to 3. Prevalence was calculated for each survey by dividing the number of parasitized damselflies by the total number of damselflies sampled per age-sex group, e.g., adult males, teneral males. Significant differences, within a survey, between adult males and females (1 asterisk), or between adults and tenerals belonging to the same gender (2 asterisks), are denoted.

survey	date	age	sex	N	prevalence %	95 % CI
1	15 June 1997	adult	female	32	18.7	33.0-67.0*
			male	33	0.0	0.0-0.0*
2	7 July 1997	adult	female	49	30.6	17.7-43.5
			male	53	41.5	28.2-54.7**
		teneral	female	49	16.3	6.0-26.6
			male	47	14.9	4.8-25.0**
3	23 July 1997	adult	female	76	44.7	33.5-55.9**
			male	41	58.5	43.5-73.5**
		teneral	female	19	10.5	3.3-24.3**
			male	7	14.3	11.6-40.2**

Grooming in relation to colonisation by mites

I used a matched paired design to test the prediction that damselflies increase the amount of time they spend grooming when being colonised by low or high numbers of *L. americana* mites. Matched damselflies were the same sex and age and had similar abundance of *Arrenurus* mites and did not differ in their body size (Appendix 2). For experiments with low numbers of colonising mites, I found that teneral females colonised by 4.4 ± 0.62 *L. americana* mite larvae increased the amount of time they spent grooming their eyes and legs when compared to control teneral females (one-tail $p=0.002$; Table 1.5). Teneral males, adult males and adult females exposed to low numbers of *L. americana* mite larvae did not show greater grooming rates over controls (all one-tail $p \geq 0.20$; Table 1.5).

In the experiment where adults were exposed to high numbers of mite larvae (ca. 12 mites per damselfly), I found that both parasitized adult males and adult females significantly increased the amount of time spent grooming (both one-tail $p \leq 0.001$; Table 1.5). I also found that the proportion of *L. americana* mites that were successful in attaching to damselflies ranged from 0% to 80% (median 34.5%) for adult females and from 9% to 80% (median 40%) for adult males. Thus, many adults are relatively effective in removing crawling mite larvae.

Table 1.5: Grooming response of *E. ebrium* damselflies parasitized with low and high levels of *L. americana*. Shown are the sample size (N), median time spent grooming, and the 25th and 75th interquartile range (IQR). Results of the Wilcoxon matched pairs test comparing the total amount of time control (C) and parasitized (P) damselflies spent engaged in grooming behaviours within an age-sex group are shown. The p-values shown in the table are the actual values which were compared to the Bonferroni corrected p-values to determine significance. Bold p-values are significant after applying the sequential Bonferroni correction procedure.

Grooming experiment with colonising <i>L. americana</i> mite larvae								
number of mites	age	sex	treatment	N	median (sec.)	IQR	Z	1-tailed p
low	teneral	male	P	20	0.0	0.0-13.5	0.80	0.21
			C	20	0.0	0.0-10.0		
low	teneral	female	P	23	13.0	0.0-38.0	2.89	0.002
			C	23	0.0	0.0-0.0		
low	adult	male	P	20	3.0	1.0-14.0	0.26	0.40
			C	20	5.0	0.0-14.0		
low	adult	female	P	19	2.0	0.0-4.0	0.82	0.20
			C	19	0.0	0.0-3.0		

Table 1.5: continued

Grooming experiment with colonising <i>L. americana</i> mite larvae								
number of mites	age	sex	treatment	N	median (sec.)	IQR	Z	1-tailed p
high	adult	male	P	21	6.0	1.0-21.0	3.2	0.0005
			C	21	0.0	0.0-0.5		
high	adult	female	P	20	13.0	6.5-41.0	3.7	0.001
			C	20	0.0	0.0-1.0		

Survivorship experiments

After seeing that statistical assumptions were met, I used an ANCOVA (Sokal and Rohlf 1995) to compare survivorship between the 2 treatment groups while controlling for the potential effect of body size as a covariate. Mites were expected to decrease the survivorship of their host (Forbes and Baker 1990, 1991; Smith 1989); as such, I used a one-tailed p-value. The sequential Bonferroni correction procedure was used to correct for the multiple comparisons within each type of experiment (complete or partial food deprivation and low versus high numbers of mites). The number of *Arrenurus* mites did not differ between the control and parasitized groups, with the exception of the adult females used in the experiment in which they were completely food deprived (Appendices 3 and 4).

Among the damselflies that were completely food deprived, I did not find any significant difference in the number of half days survived by control and parasitized damselflies (Table 1.6). Among damselflies that were partially food deprived, I also did not find a significant difference in the survivorship of controls and individuals parasitized with low numbers of mites (Table 1.7). However, for the damselflies that were partially food deprived, I found that teneral males and females and adult males showed a significant or near significant reduction in survivorship, whereas adult females did not appear to be affected, if parasitized by high numbers of *L. americana* mites (Table 1.7).

However, the actual numbers of mites that successfully engorged on adult females subjected to high parasitism was actually quite low (Appendix 1).

Maturation of females

As mentioned, for females used to test the effect of *L. americana* mites on maturation time, parasitized females compared to controls had significantly fewer *Arrenurus* mites, but were the same body size (Appendix 5). Notwithstanding, control damselflies as compared to parasitized females changed to the sexually mature coloration sooner and had a significantly greater number of eggs (Table 1.8).

Table 1.6: Survivorship of completely food-deprived *E. ebrium* damselflies parasitized with low numbers of *L. americana* mites. Shown are the sample size (N), mean and standard deviations for the number of engorging *L. americana* mites, mean and standard deviations for the number half days survived and the mean and standard deviations for the covariate (wing length). The ANCOVA results comparing the number of half days survived by control (C) and parasitized (P) damselflies within each age-sex group are also given. The p-values shown in the table are the actual values which were compared to the Bonferroni corrected p-values to determine significance

age	sex	treatment	N	wing length: mean $\pm$ std.dev.	<i>L. americana</i> mites mean $\pm$ std.dev.	survival(half days): mean $\pm$ std.dev.	df	F	1-tailed p
teneral	male	P	13	11.4 $\pm$ 0.42	2.6 $\pm$ 1.3	4.5 $\pm$ 0.71	1,28	0.5	0.26
		C	18	11.4 $\pm$ 0.34	0	4.2 $\pm$ 1.2			
teneral	female	P	18	12.3 $\pm$ 0.35	1.9 $\pm$ 0.72	6.4 $\pm$ 2.1	1,37	1.2	0.36
		C	22	12.0 $\pm$ 0.45	0	5.6 $\pm$ 2.3			
adult	male	P	13	11.3 $\pm$ 0.43	1.8 $\pm$ 0.72	3.9 $\pm$ 2.0	1,24	1.3	0.37
		C	14	11.3 $\pm$ 0.40	0	3.1 $\pm$ 1.6			
adult	female	P	12	12.1 $\pm$ 0.2	2.1 $\pm$ 1.1	2.6 $\pm$ 2.0	1,28	2.1	0.08
		C	19	12.1 $\pm$ 0.39	0	3.7 $\pm$ 1.9			

Table 1.7: Survivorship of partially food-deprived *E. ebrium* damselflies parasitized with low and high numbers of *L. americana* mites. Shown are the ANCOVA results comparing control (C) and parasitized (P) damselflies within an age-sex group, and the sample size (N), means and standard deviations for the number of engorging *L. americana* mites, mean and standard deviations for the number of half days survived and mean and standard deviations for the covariate (wing length). The p-values shown in the table are the actual values which were compared to the Bonferroni corrected p-values to determine significance. Tendency towards a significant difference are denoted with an asterisk.

Low <i>L. americana</i> mite numbers									
age	sex	treatment	N	wing length: mean $\pm$ std.dev.	<i>L. americana</i> mites mean $\pm$ std.dev.	survival (half days): mean $\pm$ std.dev.	df	F	1-tailed p
teneral	male	P	21	11.6 $\pm$ 0.37	2.0 $\pm$ 1.5	5.3 $\pm$ 2.1	1,56	1.24	0.13
		C	38	11.7 $\pm$ 0.59	0	6.2 $\pm$ 2.5			
teneral	female	P	16	12.1 $\pm$ 0.42	2.2 $\pm$ 1.2	5.0 $\pm$ 2.0	1,45	0.07	0.40
		C	32	12.1 $\pm$ 0.44	0	5.1 $\pm$ 2.1			
adult	male	P	8	11.5 $\pm$ 0.41	2.0 $\pm$ 1.5	4.1 $\pm$ 2.6	1,19	0.01	0.45
		C	14	11.4 $\pm$ 0.44	0	4.1 $\pm$ 1.9			
adult	female	P	21	12.3 $\pm$ 0.33	3.5 $\pm$ 1.9	4.7 $\pm$ 2.2	1,33	1.90	0.09
		C	15	12.3 $\pm$ 0.51	0	5.7 $\pm$ 1.7			

Table 1.7 continued

High <i>L. americana</i> mite numbers									
age	sex	treatment	N	wing length: mean±std.dev.	<i>L. americana</i> mites mean±std.dev.	survival (half days): mean±std.dev.	df	F	1-tailed p
teneral	male	P	16	11.6±0.33	6.9±3.1	5.1±1.6	1,29	5.5	0.013*
		C	16	11.6±0.34	0	6.1±0.62			
teneral	female	P	16	12.3±0.28	5.7±3.3	5.6±1.9	1,29	2.4	0.06*
		C	16	12.2±0.27	0	6.6±1.6			
adult	male	P	17	11.8±0.32	3.7±2.2	5.8±1.4	1,31	4.2	0.02*
		C	17	11.7±0.34	0	6.8±1.5			
adult	female	P	26	12.2±0.41	3.6±1.8	6.1±1.6	1,49	0.03	0.43
		C	26	12.4±0.40	0	6.3±1.8			

Table 1.8: Maturation and the median number of eggs produced by *E. ebrium* female damselflies parasitized with high levels of *L. americana* mite larvae.

Shown are the sample size (N), median number of eggs, and the 25th and 75th interquartile range (IQR) for the 2 treatment groups. The Mann Whitney U test results comparing the number of eggs in control (C) and parasitized (P) damselflies are also shown. The bold p-value indicates that this difference is significant at the 95% confidence level.

treatment	N	mature coloration	median number of eggs	IQR	U	p
P	4	no	4.0	0.0-50.5	0.0	0.02
C	4	yes	209.0	183.5-241.5		

Behavioural responses of adult males

I did not find any significant difference in the proportion of time that control males and parasitized males (with low numbers of *L. americana* mites beginning to engorge) spent in reproductive and non-reproductive behaviours, after being presented with the male model (Table 1.9). I also did not find any differences in the presentation on which successful tandem with the female model occurred (Table 1.9). Furthermore, I found no significant differences in the behavioural responses to the male or female model by control and parasitized males (with either low or high numbers of partially-engorged *L. americana* mites; Tables 1.10 and Table 1.11). All males compared in experiments had similar numbers of engorging *Arrenurus* mites and showed no differences in body size (Appendix 6).

Behavioural responses of adult females and teneral

I found that there was no significant difference in the proportion of time that the control and parasitized damselflies spent engaged in non-reproductive behaviours (flight, foraging flight or in inspecting the male model) for either male teneral, female teneral or adult females (Table 1.12) when parasitized by high numbers of partially-engorged *L. americana* mites. All individuals compared in experiments had similar numbers of engorging *Arrenurus* mites and showed no differences in body size (Appendix 7).

Table 1.9: Behavioural response of control (C) and parasitized (P) adult male damselflies to the models. Damselflies had low numbers of *L. americana* mites beginning to engorge. Shown are the sample size (N), median proportion of time spent engaged in a behaviour, 25th and 75th interquartile range (IQR) and Wilcoxon matched pairs test results. With respect to the behaviour 'successful tandem' the median value represents the median score obtained by the focal male damselfly, (score of 1 to 3: tandem achieved, score of 4: tandem was not achieved. The actual p-values are shown in the table and were compared to the Bonferroni corrected p-values to determine significance.

variable	model	treatment	N	median	IQR	Z	p
flight	male	P	21	0.05	0.0-0.20	0.36	0.72
		C	21	0.02	0.0-0.14		
inspecting	male	P	21	0	0.0-0.0	0.10	0.92
		C	21	0	3 0.0-0.34		
foraging flight	male	P	21	0.60	0.0-0.94	0.06	0.95
		C	21	0.61	0.0-0.90		
mate searching	male	P	21	0	0.0-0.0	-	-
		C	21	0	0.0-0.0		
misdirected sexual behaviour	male	P	21	0	0.0-0.0	-	-
		C	21	0	0.0-0.0		
successful tandem	female	P	21	4	2-4	0.47	0.64
		C	21	4	2-4		

Table 1.10: Behavioural response of control (C) and parasitized (P) adult male damselflies to the models.

Damselflies had low numbers of partially-engorged mites. Shown are the sample size (N), median proportion of time spent in a behaviour, 25th and 75th interquartile range (IQR) and the Wilcoxon matched pairs test results.

With respect to the behaviour 'successful tandem' the median value represents the median score obtained by the focal male damselfly, (score of 1 to 3: tandem achieved, score of 4: tandem was not achieved. The actual p-values are shown in the table and were compared to the Bonferroni corrected p-values to determine significance.

variable	model	treatment	N	median	IQR	Z	p
flight	male	P	29	0.10	0.04-0.30	1.8	0.07
		C	29	0.03	0.0-0.14		
inspecting	male	P	29	0.06	0.0-0.35	1.4	0.15
		C	29	0.02	0.0-0.18		
foraging flight	male	P	29	0.13	0.0-0.48	1.8	0.07
		C	29	0.38	0.0-0.74		
mate searching	male	P	29	0	0.0-0.18	1.1	0.27
		C	29	0	0.0-0.38		
misdirected sexual behaviour	male	P	29	0	0.0-0.0	0.94	0.34
		C	29	0	0.0-0.0		
successful tandem	female	P	29	4	3.0-4.0	0.12	0.91
		C	29	4	2.0-4.0		

Table 1.11: Behavioural response of control (C) and parasitized (P) adult male damselflies to the models.

Damselflies had high numbers of partially-engorged mites. Shown are the sample size (N), median proportion of time spent engaged in a behaviour, 25th and 75th interquartile range (IQR) and the Wilcoxon matched pairs test results. With respect to the behaviour 'successful tandem' the median value represents the median score obtained by the focal male damselfly, (score of 1 to 3: tandem achieved, score of 4: tandem was not achieved. The actual p-values are shown in the table and were compared to the Bonferroni corrected p-values to determine significance.

variable	model	treatment	N	median	IQR	Z	p
flight	male	P	19	0.11	0.0-0.21	0.84	0.40
		C	19	0.10	0.03-0.67		
inspecting	male	P	19	0	0.0-0.17	1.4	0.17
		C	19	0	0.0-0.07		
foraging flight	male	P	19	0.50	0.0-0.80	0.54	0.59
		C	19	0.33	0.0-0.80		
mate searching	male	P	19	0	0.0-0.0	1.1	0.27
		C	19	0	0.0-0.0		
misdirected sexual	male	P	19	0	0.0-0.0	-	-
behaviour		C	19	0	0.0-0.0		
successful tandem	female	P	19	4	4.0-4.0	1.1	0.27
(female model)		C	19	4	2.0-4.0		

Table 1.12: Behavioural response of adult females and teneral males and females to the male model. Damsel flies had high numbers of partially-engorged mites. Shown are the sample size (N), median proportion of time spent engaged in a behaviour, 25th and 75th interquartile range (IQR) and the Wilcoxon matched pairs test results comparing control (C) and high-parasitized (P) groups. The p-values shown in the table are the actual values which were compared to the Bonferroni corrected p-values to determine significance.

age	sex	variable	N	treatment	median (proportion of time)	IQR	Z	p
teneral	male	flight	15	P	1.0	0.5-1.0	0.71	0.48
			15	C	1.0	0.75-1.0		
		foraging flight	15	P	0	0.0-0.5	0.21	0.83
			15	C	0	0.0-0.25		
		inspecting	15	P	0	0.0-0.0	-	-
			15	C	0	0.0-0.0		
teneral	female	flight	17	P	0	0.0-0.44	0.30	0.78
			17	C	0	0.0-0.58		
		foraging flight	17	P	0	0.0-0.0	0.18	0.86
			17	C	0	0.0-0.17		
		inspecting	17	P	0	0.0-0.0	-	-
			17	C	0	0.0-0.0		

Table 1.12 continued.

age	sex	variable	N	treatment	median (proportion of time)	IQR	Z	p
adult	female	flight	24	P	0.98	0.07-1.0	0.10	0.92
			24	C	1.0	0.04-1.0		
		foraging flight	24	P	0	0.0-0.27	0.03	0.97
			24	C	0	0.0-0.14		
		inspecting	24	P	0	0.0-0.0	-	-
			24	C	0	0.0-0.0		

Discussion

The extent to which hosts are able to reduce the impact of their parasites is important for predicting what effects parasites will have on host individuals and populations and ultimately how these effects might transcend ecological scales (from species to communities). Such information is also important for those interested in biological control of pest species. In this study, I used a model parasite-host system to determine the extent to which hosts detect and defend against natural levels of parasitism. My main goal was to test whether hosts show adaptive life-history responses (mediated by behavioural changes) to parasites, which they can detect and which are known to have negative effects on survivorship. A first step in realising this goal was to observe the natural level of parasitism that exists and then to use these levels to challenge hosts, of different age or sex classes.

My surveys showed age biases for males and females which could be explained by repeated exposure of adults which frequent the pond's edge (where *L. americana* larvae are located) more often than teneral. These results are similar to those reported by Smith and Cook (1991) for other odonates. Adult sexes showed no difference in intensity of mites but a female bias in prevalence was detected in the first survey. In general, there is an apparent seasonal increase in intensity of *L. americana* mites for adults of both sexes.

Time spent grooming increased for adult males and females exposed to high natural levels of *L. americana* mites, and for teneral females exposed to low natural levels of parasitism. It is clear that grooming can be an effective anti-parasite tactic at least in this study (where up to 40% of mites are often removed by grooming), and in studies of vertebrates (Hart 1990). However, I am not sure why teneral females appeared so sensitive to low mite numbers. However, it is noteworthy that although both sexes and age groups survived as well as controls when parasitized by low numbers of mites, three groups (including teneral females) survived poorly when parasitized by high mite numbers. Additionally teneral females also showed delayed maturation relative to controls when parasitized by high mite numbers. Thus it appears that grooming, at least under some conditions, will greatly enhance fitness of damselflies challenged by *L. americana* mites. The grooming results are also significant because they indicate that damselflies do detect mites: an important pre-requisite for adaptive responses (see below).

Several studies have found that the hosts parasitized by water mites show decreased survivorship (e.g., Lanciani and Boyt 1977, reviewed by Smith 1988). In the present study, two features were noted. First, survivorship of *E. ebrium* damselflies was only compromised when high levels of natural parasitism were used and when damselflies were allowed to feed soon after receiving their treatment. It is expected that this regime allows mites to start engorging. Although complete food deprivation should be more serious to adult damselflies,

deprivation for several days after being colonised by mites is also quite serious. The partial food deprivation used in this study would reflect cool rainy days when damselflies are forced to forgo feeding (Forbes 1991a). It is noteworthy that adult females did not appear to be affected by high mite numbers. This lack of effect may either be due to having low numbers of mite parasitizing the females or due to the females being able to compensate for the lost of energy and nutrients e.g., by resorbing eggs. For this parasite-host system, the effects of mites on host survivorship are unlikely to translate into population effects, at least under the environmental conditions emulated in this study, because very few individuals in the population at Two-Island lake actually have high levels of parasitism by *L. americana* mites.

In addition to the decrease in survivorship, I found that parasitism by *L. americana* mites also affected female maturation. I found that the onset of maturation for teneral females appear to be delayed when females are parasitized by high numbers of mite larvae (assessed by their not attaining mature coloration and having few, if any, mature eggs): a finding that is consistent with other invertebrate-mite systems (Lanciani 1980, Lanciani and May 1982, Lanciani 1985). Studying another Limnocharid, Smith (1989) found that parasitism by *L. aquatica* greatly slowed the maturation of water striders (*Gerris comatus* and *G. alacris*). In my study, delays in maturation are unlikely to translate into population effects, because only a small proportion of teneral were parasitized (prevalence < 16.5%), and usually with a low number of *L. americana* mites (less than 2 mites).

Nonetheless, fitness of parasitized individuals could be reduced greatly and thus they should respond accordingly. Such fitness reduction for certain individuals may explain why teneral females were so sensitive in their grooming responses.

In this study I was interested in testing whether parasitized adult males reallocated energy into current reproduction to increase reproductive effort. An increase in reproductive effort may involve allocating more time to mate searching and less time to foraging. Behavioural changes are usually a sensitive test of whether an individual is affected by a factor, whether biological or non-biological (Anderson 1993). Several studies have shown that mites affect hosts, either by reducing the "alertness" of the host (Brumpt 1929, Åbro 1982) or impairing its flight abilities (Reinhardt 1996). With respect to foraging activity of their hosts, mites have been associated with increases (Forbes 1991b), decreases (Fenley 1966) and even termination (Brumpt 1929). In the present study, the mate searching effort and mating effort of adult male *E. ebrium* was unaffected by low or high natural levels of parasitism. I also found that the male and female damselflies, both adult and teneral, did not alter the proportion of time they spent engaged in non-reproductive behaviours. Thus, it appears that parasitized damselflies do not alter their time spent in various behaviours (other than grooming).

The behavioural comparisons with teneral were complicated by the fact that when presented with male models, teneral often remained inactive, groomed or rotated around on their perch (known as 'squirreling' which is an

anti-predator response; Miller 1987). These responses may be natural responses to other damselflies that occasionally prey on teneral and such responses may override any effects of mites on behaviour.

In conclusion, it appears that a first line of defence against *L. americana* mites is grooming. Once parasitized, adult damselflies show no further behavioural changes over unparasitized individuals. However, the low mite levels used in these behavioural experiments were not significantly detrimental to damselflies, and we only should have expected an effect at higher mite levels. Yet even the high mite levels, were not associated with behavioural changes of damselflies, even though high levels were known to depress survivorship. This lack of congruence between observation and prediction may reflect the inappropriateness of using damselfly models to test for a host-mediated response to parasitism as previously discussed. For example, different experiments for teneral may be needed that do not rely on responses to models. Additionally, it is possible that an adaptive host response, i.e., increase in mate-searching effort, may occur only at higher numbers of mites (with respect to grooming and survivorship, a clear dose-dependence was revealed by this study). Finally, we are still uncertain what physiological defences *Enallagma* damselflies employ against *L. americana* mites, although it has been observed that on other host species these mites completely fail to engorge, showing that a physiological defence can be effective against this mite species. One promising avenue of research is whether the sexes differ in their responses to mites: a topic which

relates to adaptive allocation and cost of parasitism and which is partly addressed in the next chapter.

Chapter 2

Variation in susceptibility to parasites and costs of parasitism: a study using a generalist parasite, *Limnochares americana* (Lundblad) and three of its host species: *Enallagma ebrium* (Hagen), *Nehalennia irene* (Hagen) and *Leucorrhinia frigida* (Selys).

Introduction

The degree to which different host individuals vary in their susceptibility to parasites is important for predicting impact of parasites on host populations and fitness of parasites (Jaenike 1996). One avenue of research receiving more study is the extent to which males and females within a host species vary in their susceptibility to parasites, their immune responses to parasites, and the costs of parasitism; inferences from such studies are being used to form the conceptual framework of the field of ecological immunology (Sheldon and Verhulst 1996).

Sex biases in parasitism are well studied in vertebrates where sex differences in exposure and immune function (caused by hormonal factors) are known to be partly or wholly responsible (Alexander and Stimson 1988, Bundy 1988, Hart 1990, Mooring and Hart 1995, Schalk and Forbes 1997). Sex biases in invertebrates also have been reported; however, their causes are not well understood (Davis 1959, Booth and Learner 1978, Robinson 1983, Lanciani 1988,

Reilly and McCarthy 1991). For example, female blackflies have a higher numbers of ectoparasitic mites than males (Davis 1959), but whether this bias results from gender differences in host physiology or behaviour is unknown.

Smith (1988) summarised the available information for sex biases in aquatic insects attacked by water mites (similar to the parasite-host systems described herein). Because many insects are colonised while they are still immature, sex biases in susceptibility tend not to be realised. This lack of bias is possibly due the fact that immature males and females do not differ greatly in their ecology: they use similar habitats, exploit similar food resources and are thus similarly susceptible to larval mites. In comparison, some water mites (such as *L. americana*) actually colonise adult insects, and here differences in ecology or immune function may be expected to result in sex biases in parasitism.

In this Chapter, I explore sex biases in susceptibility to parasites and investigate any sex differences in the costs of parasitism for three species of odonates attacked by a generalist parasite, *Limnochares americana* (Lundblad). I first interpret Cook (1991)'s results: she found that field-caught *Nehalennia irene* (Hagen) and *Leucorhinnia frigida* (Selys), respectively, showed female and male biases in numbers of attending *L. americana* mites, whereas sexes of *Enallagma ebrium* (Hagen) showed no sex biases (but also see Chapter 1). Cook (1991) argued that the oviposition behaviour of female *N. irene* and the territorial behaviour of male *L. frigida* may be responsible for the observed female and male sex bias, respectively, because mites aggregate on emergent vegetation used by

the females and males of the aforementioned species. Although male *N. irene* will perch on emergent vegetation, they are elevated above females when they contact guard them (M.R. Forbes pers. obs.): thus, any mites colonising males in these instances would have to crawl across females. In comparison to males, female *L. frigida* only occasionally visit ponds to oviposit exophytically: as such, males suffer repeated exposure to larval mites while on territorial perches, whereas females get limited exposure. I was initially interested in whether sex biases in parasitism exist for these three species studied by Cook (1991), when exposure to mites is controlled in a laboratory setting. In controlling the exposure to mites, I eliminated the effect of behavioural differences between males and females that could result in a lesser or greater degree of exposure to mites (Schalk and Forbes 1997).

I also had three other objectives in my study. First, I examined whether maximum engorgement of larvae of *L. americana* was dependent on host sex, using field-caught individuals of all three species. This comparison allowed me to determine whether mites engorged larger on one sex than on another, which might mean that individual mites were more costly to that sex. However, one problem with interpreting data from field collections is that maximum engorgement size could be affected by the number of mites attending the host (Lanciani 1984) as well as the amount of time the mite has been attached and engorging on the host. Additionally, if mites are more costly to one host sex, then survivorship of that sex may be reduced in the field and the likelihood of finding

long-lived individuals of that sex with large mites also would be reduced. For these reasons, I also chose to experimentally parasitize *E. ebrium* in the laboratory to control for timing of exposure and number of mites because I knew that this species could be housed under semi-natural conditions (see Chapter 1). Using this species, I compared mite engorgement on hosts and host survivorship, with respect to host gender, under controlled conditions. Here, I was able to compare survivorship of the sexes directly (only within-sex comparisons were made in Chapter 1) to ascertain whether mites may be more costly to one sex of *E. ebrium*.

Methods

Study species

The life cycle of *L. americana* mites is described in Chapter 1. This mite species is a generalist parasite at my study areas, infesting not only the three species already listed above, but also several species of Lestid damselflies and another dragonfly (*Nannothemis bella* (Uhler)). *N. irene* is a small damselfly, *E. ebrium* is a medium-size damselfly, and *L. frigida* is a medium-sized dragonfly (see Walker 1953 for species descriptions). All 3 species inhabit beaver ponds and bogs that also serve as habitat for *L. americana*.

Study sites

In June and July 1997, I collected *L. frigida* and *E. ebrium* from Two-Island Lake and *N. irene* from Indian Lake Bight, Barb's Marsh, and Hebert's Bog. I collected egg masses of *L. americana* from Hebert's Bog. All sites are located < 15 km west of the Queen's University Biological Station (see Chapter 1 for its location). The transport of damselflies and dragonflies as well as the collection and of *L. americana* mite eggs and rearing of larvae followed the protocols described in Chapter 1.

Experimental exposure of male and female odonates to mite larvae

For the first experiment, examining colonisation by mite larvae in relation to host sex, individuals of all 3 species were collected and then were first challenged with a set number of mites before being placed in a plastic cup (as described in Chapter 1 for *E. ebrium*). In total, I challenged each of 52 mature male and 53 mature female *N. irene* with 3-6 *L. americana* larvae, each of 49 mature male and 48 mature female *E. ebrium* with 4-10 larvae, and each of 21 mature male and 20 mature female *L. frigida* with 4-11 larvae. The exact number of mites presented to each individual was recorded. The protocol for parasitizing hosts with mites was the same for all odonates, and as described in Chapter 1 for *E. ebrium*. Once all individuals had been parasitized and placed in individual cups, I moved the cups to a dim, cool room to simulate natural conditions of food deprivation and periods of low activity in the field (mean temperature =16.9°C, range: 14.0 -

19.5°C). I checked the individuals every 12 h for mortality. Upon death, I recorded the number of attached *L. americana* mites and calculated the proportion of successfully attached mites as number attached/number presented. I did not compare the sexes with respect to survivorship because they may respond differently to conditions of housing (especially *L. frigida* males which are active flyers in the field, N.J. Léonard pers. obs.). Any differences in survivorship could be due to different responses to housing rather than to differential costs of parasites.

Engorgement size of L. americana mite larvae on male and female odonates

In the field study I examined the maximum size of engorging *L. americana* on field-caught individuals of both sexes, for each of the 3 odonate species (collected in July and August 1997). I examined individuals that were presently parasitized only by *L. americana* to exclude the possibility that interspecific competition between mites may reduce engorgement on some host individuals. After transport to the laboratory, individuals were placed in plastic cups for holding (see description in Chapter 1). Within 24 hours, the largest mite per individual was carefully detached using insect pins and transferred to a petri dish. Each mite, and a 1 mm stage micrometer (used for scale) was sketched using a Wild M3 microscope with a drawing tube. Using digital callipers, I measured the maximum width of each individual mite 3 times and used averages in analyses; this reduced the effect of measurement error (Leung and Forbes 1997).

In the laboratory experiment performed using *E. ebrium*, I compared engorgement size of the mite larvae on male and female teneral *E. ebrium* (collected 17 June 1997). I controlled both the time at which colonisation occurred, the number of *L. americana* mites colonising the damselfly, and the level of parasitism by *Arrenurus* mites. After allowing mites to colonise individuals, I released damselflies into an insectarium (17 June 1998), where they could forage (as described in Chapter 1). After 3 days, I recaptured 8 males and 13 females each with 1-4 engorging *L. americana*. These damselflies were kept overnight in individual plastic holding cups and killed by decapitation the following day. Engorged mites were then detached from damselflies using insect pins, and drawn along with a micrometer scale using a drawing tube attached to a Laborolux® K microscope. The drawings and the scale were scanned into the computer using a Hewlett Packard ScanJet 3p and the 1994 HP pictureplace version 1.0 software. The area and width of each mite were then measured using Jandel Sigma Scan image software. I made 3 measurements for width and area and used averages in my analyses. As before, only the largest mite per damselfly was measured.

Survivorship differences between male and female E. ebrium

I collected 27 mature male and 31 mature female *E. ebrium*. On each collection date, I assigned male and female damselflies to either the control or parasitism treatment. Each individual within a treatment group was matched to an individual of the opposite sex for numbers of *Arrenurus* mites. Male and female *E. ebrium* assigned to the parasitism treatment received 4-6 mite larvae. After receiving their treatment, damselflies were housed individually in plastic cups and placed in a cool, dim room (mean temperature =18.7°C, range: 15.0-22.0°C). I checked the damselflies every 12 h until death. Upon death, I recorded the number of half days survived, the number of engorging *L. americana* mites and I measured forewing lengths of the damselflies (cf. Chapter 1).

Statistical methods

All the statistical analyses were done using Statistica (Statsoft Inc. 1994). Parametric test were used whenever possible, but when assumptions were violated, the equivalent non-parametric tests were used.

Results

Experimental exposure of male and female odonates to mite larvae

Similar proportions of *L. americana* larvae successfully attached on adult male and female *N. irene*, as well as on adult male and female *E. ebrium* (Table 2.1). However, I found significantly higher attachment of *L. americana* larvae on female as compared to male *L. frigida* ($p = 0.01$; Table 2.1).

Engorgement size of L. americana mite larvae on male and female odonates

In the field experiment I found that there was no significant difference in the numbers of *L. americana* mites engorging on field-caught males and females for any species used in this experiment (Appendix 8). I also found no differences between the sexes in the size of the largest mite for any of the three species based on field-collected individuals, although sample sizes for *L. frigida* were quite low (Table 2.2). In the laboratory experiment in which I parasitized *E. ebrium* with *L. americana* mites, I found that mite area was greater on female than male hosts, after controlling for body size of host by using an ANCOVA ($p=0.01$; Table 2.3). The experimentally parasitized *E. ebrium* male and female did not differ in the number of engorging *Arrenurus* or *Limnochares* mites (Appendix 9).

In the laboratory experiment, I relied only on average area because I found that, in measuring the width with a computer interface, a different orientation of

the partially-engorged mites greatly reduced the repeatability of obtaining widths accurately (due to using different reference points along the irregular outline of soft-bodied mite species like *L. americana*). Thus, the average area is probably a better indicator of engorgement size for *L. americana* because it is less affected by orientation.

Survivorship differences between male and female E. ebrium

After square-root transforming the number of half-days survived to meet the assumptions of normality, I found that neither sex ($F_{(1,53)}=0.004$, $p=0.95$; Table 2.4) nor treatment ($F_{(1,53)}=0.27$, $p=0.60$; parasitized or control) affected the survivorship of *E. ebrium* damselflies (after controlling for body size). However, there was a nearly significant interaction term ($p=0.07$) between sex and treatment, indicating that parasitized females tended to have a decrease in survivorship in comparison to parasitized males. I controlled for body size, measured by wing lengths, because body sizes differs between the sexes (although there is overlap; Walker 1953), and because body influences survivorship for *E. ebrium* within a sex (Forbes and Baker 1991).

These results might suggest that female *E. ebrium* may suffer a higher cost of parasitism by the larger engorging mites (Table 2.4) However, because I found that parasitized females had more *Arrenurus* than parasitized males (Appendix 10), although they had similar numbers to unparasitized control females, this difference in survivorship should be interpreted with caution.

Thus taken together, there was only weak evidence that parasitism by *L. americana* (at least at the levels used in this experiment, Table 2.4) was more costly to one sex.

Table 2.1: The proportion of *L. americana* larvae attaching to male and female odonates. Shown are the sample size (N), median proportion of mite attached, the 25th and 75th interquartile range (IQR), and the Mann-Whitney U-test results. P-values in bold are significant.

species	year	sex	N	median proportion of mites attached	IQR	U	p
<i>N. irene</i>	1997	male	52	0.25	0.00-0.37	1728.0	>0.25
		female	73	0.33	0.00-0.50		
<i>E. ebrium</i>	1997	male	49	0.37	0.20-0.53	1129.5	>0.50
		female	48	0.31	0.20-0.60		
<i>L. frigida</i>	1997	male	21	0.14	0.00-0.27	149.5	=0.010
		female	20	0.31	0.25-0.44		

Table 2.2: Field experiment comparing engorgement size of *L. americana* on field-parasitized male and female odonates. Shown are the sample size (N), mean and standard deviation of the average width (mm) of *L. americana* mite larvae. The average width is calculated from measuring the width of the largest mite found on each individual odonate. The ANOVA results comparing mite engorgement size between males and females within an odonate species are also shown.

species	sex	N	average mite width mean±std.dev.	F	df	p
<i>N. irene</i>	male	35	0.38±0.04	1.4	1, 68	0.25
	female	35	0.39±0.04			
<i>E. ebrium</i>	male	32	0.41±0.05	0.36	1, 63	0.55
	female	33	0.40±0.04			
<i>L. frigida</i>	male	16	0.39±0.05	0.44	1, 16	0.52
	female	2	0.42±0.01			

Table 2.3: Laboratory experiment comparing engorgement size of *L. americanus* on laboratory-parasitized male and female *E. ebrius*. Shown are the sample size (N), mean and standard deviation, and the engorgement size (average area, mm^2)² of *L. americanus* larvae 3 days post laboratory colonisation. The average area is calculated by measuring the area of the largest mite per each individual odonate. The ANCOVA results comparing engorgement size between males and females, using wing length (mm) as the covariate to correct for damselfly body size, are also given. The bold p-value indicates that the difference between the sexes is significant.

variable	male		female		ANCOVA results		
	N	mean±std.dev.	N	Mean± std.dev.	df	F	p
mite area	8	0.11±0.03	13	0.17±0.03	1,18	7.96	0.01
damselfly wing	8	11.4±0.50	13	12.4±0.43			
length							

* If I used the average mite width and performed a simple ANOVA, as was done in the field experiment, I still detect a significant difference in mite engorgement size between male and female *E. ebrius* ($F_{(1, 19)}=4.60$, $p=0.04$).

Table 2.4: Survivorship of control (C) and parasitized (P) male and female *E. ebrium*. . Shown are the mean and standard deviation of engorging *L. americana* mites (mites), the square root transformation of the number of half days survived, wing length, and 2-way factorial ANCOVA results are shown. The square root back transformation of the mean and the upper maximum and lower minimum standard deviation (min.-max.) of the number of half days survived (bk.-tr.; Elliot 1977) are also shown. An asterisk denotes a tendency towards significance.

descriptive data				2-way factorial ANCOVA results						
treatment	sex	N	number of mites	number of half days survived	bk.-tr. mean (max.-min)	wing length (mm)	effect	df	F	P
P	male	13	1.7±0.72	1.9±0.59	4.0 (6.6-2.1)	11.3±0.43	sex	1,53	0.004	0.95
	female	12	1.9±1.0	1.5±0.63	2.6 (4.9-1.1)	12.1±0.23	trt	1,53	0.27	0.60
C	male	14	N/A	1.7±0.49	3.1(5.0-1.7)	11.3±0.40	sex*trt	1,53	3.3	0.07*
	female	19	N/A	1.9±0.52	3.9 (6.1-2.2)	12.1±0.39				

Discussion

The most salient findings of my study are threefold: first, the sex biases, reported by Cook (1991), in parasitism by *L. americana* for field-caught odonates are not reproduced when exposure is controlled for in the laboratory; second, differences do not exist between sexes of hosts in terms of engorgement sizes of *L. americana* when sampled in the field; third, for one host species, *L. americana* mites tend to engorge to larger sizes on females than on males when exposure and timing of engorgement is controlled under laboratory and semi-natural conditions. I will discuss the relevance of each of these findings in turn.

First, I found support for Cook's (1991) arguments that female and male biases in parasitism for *N. irene*, and *L. frigida*, respectively, are due to differential exposure in the field between the sexes, because when I controlled for exposure, these sex biases were not reproduced in the laboratory. In fact, for *L. frigida*, females were actually more likely to be colonised in the laboratory even though there was a male bias for field-caught individuals. These results suggest that differential exposure in the field rather than differential immunity is a more likely explanation for the natural sex biases observed.

Exposure of female to larval mites is limited compared to the male *N. irene*. As Cook (1991) noted, oviposition in *N. irene* can last up to 1 hour, and it is during this time that females, but not males, come into contact with floating vegetation

which can carry *L. americana* mites (Walker 1953, Smith 1988). As mentioned above, adult male *L. frigida* can spend a large proportion of their time perched on vegetation occupied by mites. On the other hand, female *L. frigida* only come to the pond to copulate and oviposit (copulation ranges from 170 to 610 seconds; Cook 1991) and occurs in vegetation surrounding a marsh, where mites are not present. Furthermore, females oviposit their eggs exophytically by dipping the tip of their abdomens on the surface of the water and releasing eggs while in flight (Walker 1953). It is interesting that the sex most exposed to mites in the field (average loads of 12.0 mites per host; Cook 1991) actually appears less susceptible under controlled conditions, which is consistent with the idea that males may have been selected more so than females (with average loads of 0.4 mites per host; Cook 1991) to express resistance because of their higher encounter rates. Differential susceptibility also has been reported in comparisons of species that differ in their temporal overlap with parasites, when temporal barriers are removed under controlled conditions (Smith 1988).

It is important to note that both my study and Cook's (1991) study did not measure longevity of males and females in the field. It is possible that one sex survives longer in the field (in fact, there is preliminary evidence that this is the case for *N. irene* females based on laboratory work, N.J. Léonard unpubl. data) and could accumulate more mites simply because it lives longer. This is the case in comparing heavily-parasitized male *L. frigida* to lightly-parasitized males (Smith and Cook 1991), where males observed to live longer actually had more *L.*

americana mites probably due to their repeated exposure. It is also interesting that for *L. frigida*, female-biased dispersal occurs (Cook 1991). Thus, adults sampled at places where mites are located will more likely include males that also emerged from those sites than females.

As mentioned, no sex biases in engorgement sizes of mites were observed for field-collected hosts of any species. Providing that individual mites exert the same cost to hosts, then female *N. irene* and male *L. frigida* would be more affected by *L. americana* mites than conspecifics of the opposite sex simply because female *N. irene* and male *L. frigida* accumulate more mites. This would not be the case for *E. ebrium* which showed no sex biases in degree of parasitism nor engorgement sizes of mites for field-collected individuals. However, it was interesting that mites under controlled conditions actually engorged more on females than on males, even after correcting for the effects of body size. As such, individual mites may exact larger costs from female *E. ebrium* than from males, a result that would not be inferred from field-collected specimens. This cost, however, does not appear that great (at least at the natural levels of parasitism observed in this study), although there was a tendency for females parasitized by *L. americana* mites to survive less well than control females and males, and parasitized males. Clearly more work is needed to test whether differential costs exist.

In conclusion, several factors may come into play in determining the origin of a sex bias in the field and/or laboratory experiments (e.g., exposure, grooming behaviour, immune responses). Most often, sex-biases observed in invertebrate

hosts are not reproduced in the laboratory and suggest that these biases are linked to behavioural differences between the sexes (Smith 1988). However, laboratory studies also indicate other factors that may exaggerate or lessen biases, such as differential longevity or apparent differences in fixed-natural immunity. These laboratory studies also can show differential costs based on engorgement sizes not seen in field-collected individuals.

General Conclusion and Prospectus

In the first chapter, parasitised adult male damselflies were not observed to increase their reproductive effort in response to the high numbers of *L. americana* mite larvae used in this study. The apparent absence of a host-mediated response by the parasitised adult male damselfly does not exclude the possibility of a host-mediated response in this damselfly-mite association. Based on the results of the grooming and survivorship experiment, I found that there exists a clear dose dependency between the number of mite larvae per damselfly host and whether the damselfly responded to mites by increasing grooming, or whether the host's survivorship was reduced. Thus, there is a possibility that a host-mediated response, either as an increase in reproductive effort or another type of response, might occur at higher parasitism levels than those used in this study.

The findings of this study suggests that at higher natural levels of parasitism (greater than 4 mites per host) damselflies may respond to the presence of the mites. It would be of interest to test whether the adult male changes its reproductive strategy, i.e., parasitised males wait by the pond to capture an unguarded ovipositing female versus competing for unmated females. A host response also may be detected by the adult female; it would be revealing to look at whether the adult female increased her reproductive effort by increasing the number of eggs produced, oviposited, or the size of those eggs

(larger eggs may increase hatching success). It also may be of interest to investigate the possible occurrence of other host responses that do not involve increasing reproductive effort, which may be used by the parasitised damselflies to decrease the impact of the parasite on host fitness, such as increasing the amount of time spent foraging to offset nutrient lost to the mite, or the occurrence of an host immunological response to the mite larvae, i.e., melanization of the mite's stylostome.

In the second chapter, I found that the sex biases observed in the field (female bias in *N. irene* and male bias in *L. frigida*) were most likely due to differential exposure in the field as suggested by Cook (1991). However, under controlled conditions, my laboratory experiments revealed a female sex bias in mite attachment for *L. frigida*, which may be due to differences in behavioural or immunological response between the sexes, and that mite larvae engorged to a larger size on female *E. ebrium*. The female bias in mite attachment on *L. frigida* may be explained by males being more exposed to mites in the field and, as such, have evolved increased immunity under controlled conditions. Differences in mite engorgement on *E. ebrium* suggest that males and females may be differentially affected by the engorging mite larvae although a sex bias in mite intensity was not observed in field populations. Based on these results it would be interesting to test whether mites differentially affect life history traits of male and female hosts (i.e., survivorship) and whether male and female hosts differ in their immune response to the mite larvae. If the sexes are found to differ with

respect to the effect of the engorging mite larvae, this may influence our expectation of which sex of a host species may engage in a host-mediated response to the mite larvae. Furthermore, if males and females of a host species are differentially affected by a parasite due to a sex bias in parasitism or the presence of a host-mediated response it would be necessary to take into account these 2 factors before determining the effectiveness of parasites as a biological control agent for that host population.

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Appendix 1: The median number of *L. americana* mites parasitizing damselflies assigned to the low-parasitism and high-parasitism treatments. Shown are the parasitism treatments, median, and the 25th and 75th interquartile range (IQR) per age-sex group for each experiment. Where adults, tenerals, males and females are represented by A, T, M, and F, respectively.

experiment	parasitism treatment	sex-age	median	IQR 25th-75th
complete food deprived	low	A-M	2.0	1.0-2.0
survivorship	low	A-F	2.0	1.0-2.5
	low	T-M	3.0	1.0-3.5
	low	T-F	2.0	1.0-2.0
partially food deprived	low	A-M	2.0	1.5-3.0
survivorship	low	A-F	4.0	2.0-4.5
	low	T-M	1.0	1.0-2.0
	low	T-F	2.0	1.0-2.5
	high	A-M	3.0	2.0-4.0
	high	A-F	3.5	2.0-5.0
	high	T-M	7.0	4.5-9.5
	high	T-F	4.5	3.5-7.5

Appendix 1: continued.

experiment	parasitism	sex-age	median	IQR
	treatment			25th-75th
maturation	high	A-F	4.5	2.5-5.5
grooming	low	A-M	4.0	4.0-4.5
	low	A-F	4.0	4.0-5.0
	low	T-M	4.0	4.0-4.5
	low	T-F	4.0	4.0-4.5
	high	A-M	12.0	10.0-16.0
	high	A-F	10.0	10.0-12.0
behavioural response of males with mites beginning to engorge	low	A-M	2.0	1.0-2.0
behavioural response of males with mites partially-engorged	low	A-M	2.0	1.0-2.0
	high	A-M	3.0	2.0-4.0
behavioural response of adult female and teneral	high	A-F	3.0	2.0-5.0
	high	T-M	7.0	4.0-10.0
	high	T-F	5.0	3.0-7.5

Appendix 2: Grooming experiments on *E. ebrium* damselflies using low and high numbers of *L. americana* mite larvae. The comparisons are made between treatment groups, control (C) and parasitized (P), for adult (A) and teneral (T), male (M) and female (F) damselflies. Show are the sample size (N), mean and standard deviation of wing length and the number of *Arrenurus* mites. The results of the paired T-test comparing the number of *Arrenurus* mites and the wing length between treatment groups.

experiment	age-sex	trt	N	number of <i>Arrenurus</i>				wing length (mm)			
				mean $\pm$ std. dev.	df	t	p	mean $\pm$ std. dev.	df	t	p
low	T-M	P	20	8.2 $\pm$ 3.9	19	0.78	0.44	11.7 $\pm$ 0.43	18	0.98	0.34
		C	20	8.9 $\pm$ 4.5				11.5 $\pm$ 0.46			
	T-F	P	23	6.9 $\pm$ 3.8	22	1.55	0.13	12.3 $\pm$ 0.47	23	1.02	0.32
		C	23	8.1 $\pm$ 4.2				12.4 $\pm$ 0.40			
low	A-M	P	20	8.4 $\pm$ 7.3	19	0.29	0.77	11.5 $\pm$ 0.36	19	0.42	0.68
		C	20	8.1 $\pm$ 7.4				11.5 $\pm$ 0.36			
	A-F	P	19	6.3 $\pm$ 4.8	18	0.71	0.49	12.2 $\pm$ 0.42	18	.12	0.90
		C	19	7.0 $\pm$ 4.8				12.2 $\pm$ 0.33			
high	A-M	P	21	10.5 $\pm$ 5.4	40	0.49	0.62	11.8 $\pm$ 0.29	40	0.49	0.62
		C	21	9.7 $\pm$ 5.1				11.8 $\pm$ 0.39			
	A-F	P	20	10.7 $\pm$ 5.5	36	0.48	0.63	12.6 $\pm$ 0.26	36	0.39	0.70
		C	20	9.9 $\pm$ 5.6				12.7 $\pm$ 0.33			

Appendix 3: Survivorship experiments in which damselflies were completely food deprived, and parasitized with low numbers of *L. americana* mites. The Student T-test results comparing the number of *Arrenurus* mites engorging on parasitized (P) and control (C) *E. ebrium* damselflies, the sample size (N), mean and standard deviation of *Arrenurus* mites are shown. The bold p-values indicate a significant difference between treatment group.

age	sex	treatment	N	mean±std.dev.	df	t	p
teneral	males	P	13	7.6±6.2	29	0.44	0.66
		C	18	8.4±3.6			
teneral	females	P	18	8.7±5.3	38	0.95	0.35
		C	22	7.4±3.3			
adult	males	P	13	5.5±4.6	25	1.03	0.31
		C	14	7.6±5.8			
adult	females	P	12	10.6±5.8	30	2.69	0.01
		C	20	6.0±3.8			

Appendix 4: Survivorship experiments in which damselflies were parasitized with low and high levels of *L. americana* mites, and were partially food deprived. Shown are the Student T-test results comparing the number of *Arrenurus* mites engorging on adult (A), teneral (T), male (M) and female (F) *E. ebrium* damselflies, the treatment group, parasitized (P) or control (C), sample size (N), mean and standard deviation of *Arrenurus* mites.

experiment	age	sex	treatment	N	mean $\pm$ std.dev.	df	t	p
low	T	M	P	21	8.0 $\pm$ 6.7	57	0.10	0.92
			C	38	8.1 $\pm$ 5.2			
low	T	F	P	16	8.1 $\pm$ 3.8	46	0.21	0.84
			C	32	8.3 $\pm$ 4.8			
low	A	M	P	8	3.6 $\pm$ 5.4	20	0.64	0.53
			C	14	2.3 $\pm$ 3.9			
low	A	F	P	21	4.4 $\pm$ 4.0	34	0.47	0.64
			C	15	5.2 $\pm$ 6.4			
high	T	M	P	15	6.7 $\pm$ 4.1	28	0.44	0.66
			C	15	7.3 $\pm$ 4.2			
high	T	F	P	17	11.3 $\pm$ 5.8	32	0.11	0.91
			C	17	11.0 $\pm$ 6.4			
high	A	M	P	19	8.2 $\pm$ 3.9	36	0.18	0.85
			C	19	8.0 $\pm$ 3.0			
high	A	F	P	24	9.0 $\pm$ 5.5	46	1.4	0.15
			C	24	11.3 $\pm$ 5.3			

Appendix 5: Maturation experiment on *E. ebrium* females using high levels of *L. americana* mites. Shown are the median and the 25th and 75th interquartile range and Mann-Whitney U-test results per treatment group, parasitized (P) or control (C), for forewing length, and the number of engorging *Arrenurus* mites. A bold p-value indicates that there is a significant difference between treatment groups.

variable	treatment	median	IQR	Z	p
<i>Arrenurus</i>	P	3.5	3.0-4.5	2.3	0.02
	C	8.0	8.0-9.0		
forewing length (mm)	P	12.5	12.3-12.9	0.58	0.56
	C	12.4	12.0-12.7		

Appendix 6: Behavioural response of adult male *E. ebrium* to male and female models. The experiments on the behavioural response of adult males include: adult males with low numbers of mites beginning to engorge (LS), low numbers of partially-engorged mites (LP) and high numbers of partially-engorged mites (HP). Shown are the treatment group, parasitized (P) or control (C), sample size (N), mean and standard deviation of *Arrenurus* mites, and wing length. The Student T-test results comparing the number of *Arrenurus* mites and wing length between treatment groups within each experiment.

		number <i>Arrenurus</i> mites					wing length (mm)			
exp.	treatment	N	mean±std.dev.	df	t	p	mean±std.dev.	t	df	p
LS	P	21	8.2±4.9	40	1.06	0.30	11.5±0.25	0.14	20	0.89
	C	21	6.7±4.1				11.5±0.46			
LP	P	29	7.7±4.2	56	0.71	0.48	11.0±2.1	0.30	32	0.76
	C	29	8.5±4.6				10.6±2.9			
HP	C	19	8.0±3.0	36	0.18	0.85	11.7±0.39	0.45	36	0.65
		19	8.2±3.9				11.7±0.33			

Appendix 7: Behavioural response of female adults and teneral, parasitized with high levels of *L. americana* mites to the male model. Comparisons are made between treatment groups, parasitized (P) or control (C), for adult females (A-F), teneral female (T-F) and teneral male (T-M). Shown are the sample size (N), mean and standard deviation of the number of *Arrenurus* mites, and wing length. The Student T-test results comparing the number of *Arrenurus* mite and wing length between treatment groups.

sex	N	trt	number of <i>Arrenurus</i> mites				wing length (mm)			
			mean $\pm$ std.dev.	df	t	p	mean $\pm$ std.dev.	df	t	p
T-M	15	P	6.7 $\pm$ 4.1	28	0.44	0.66	11.6 $\pm$ 0.34	28	0.44	0.66
	15	C	7.3 $\pm$ 4.2				11.6 $\pm$ 0.34			
T-F	17	P	11.3 $\pm$ 5.8	32	0.11	0.91	12.4 $\pm$ 0.35	31	1.64	0.11
	17	C	11.0 $\pm$ 6.4				12.2 $\pm$ 0.28			
T-F	24	P	9.0 $\pm$ 5.5	46	1.4	0.15	12.5 $\pm$ 0.41	46	0.66	0.51
	24	C	11.3 $\pm$ 5.3				12.4 $\pm$ 0.42			

Appendix 8: Experiment comparing the engorgement size of *L. americana* mites found on field-caught male and female odonates. Given are the sample size (N), mean and standard deviations. The median number of *L. americana* mites on field caught male (M) and female (F) odonates were compared using the Student T-test.

species	sex	N	mean $\pm$ std.dev.	t	df	p-value
<i>E. ebrium</i>	M	32	2.2 $\pm$ 1.5	0.61	63	0.54
	F	33	2.5 $\pm$ 1.9			
<i>N. irene</i>	M	35	2.9 $\pm$ 1.8	1.3	68	0.21
	F	35	2.4 $\pm$ 1.6			
<i>L. frigida</i>	M	16	4.9 $\pm$ 11.1	0.48	16	0.64
	F	2	1.0 $\pm$ 0.0			

Appendix 9: Experiment comparing the engorgement size of *L. americana* mites engorging on laboratory parasitized male and female *E. ebrium* damselflies.

Given are the sample size (N), median and the 25th and 75th interquartile range (IQR). The median number of *Arrenurus* and *L. americana* mites on male (M) and female (F) *E. ebrium* were compared using the Mann-Whitney U test.

mite species	sex	N	median	IQR (25th-75th)	U	p-value
<i>Arrenurus</i>	M	8	12.0	7.5-20.5	49.5	0.86
	F	13	12.0	8.0-15.0		
<i>L. americana</i>	M	8	3.0	1.5-3.0	36.0	0.25
	F	13	2.0	1.0-2.0		

Appendix 10: Survivorship experiments for completely food deprived male and female *E. ebrium*. Mean number of *Arrenurus* mites engorging on males (M) and females (F) odonates used in the survivorship experiment. Shown are the treatment groups (parasitized, P, control, C), sample size (N), mean and standard deviation of the number of *Arrenurus* mites engorging on male (M) and female (F) odonates and the Student T-tests results comparing the number of *Arrenurus* mites on males and females. P-values in bold indicate a significant difference between genders.

species	treatment	sex	N	<i>Arrenurus</i> mean±std.dev.	df	t	p
<i>E. ebrium</i>	P	M	13	5.5±4.6	23	2.4	0.02
		F	12	10.6±5.8			
	C	M	14	7.6±5.8	32	1.0	0.32
		F	20	6.0±3.8			

IMAGE EVALUATION TEST TARGET (QA-3)

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