Parasitism and survival in a damselfly: does host sex matter?

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We present experimental data on the survivorship of damselflies infested by parasitic water mites from a population in field cages. In addition, we show correlative laboratory data under simulated severe weather conditions. In the manipulative experiment, parasitized females' individual condition, which was measured as weight at emergence, was an important determinant of survival under field conditions. In contrast, such a relationship did not occur in males and unparasitized females. It was found in the laboratory experiment that water mites as well as weight at emergence both contributed significantly to the reduced survivorship of male and female damselflies. It was concluded that the impact of parasitism depends on environmental conditions and that host sexes differ in their responses to parasitism. This is discussed in the light of immunocompetence in invertebrates.

Keywords: condition-dependent survival; sex-specific mortality; Odonata; water mites

1. INTRODUCTION

Parasites are known to influence host fitness in different ways (Lehmann 1993). One of the most important consequences for hosts is an increase in their mortality (e.g. Lanciani 1975; May & Anderson 1978; Polak 1996; Van Sambeek & Wiesner 1999). Jaenike *et al.* (1995) pointed out that the influence of parasitism in experiments under natural conditions has rarely been studied.

An increase in mortality can be caused by the drain of nutrients or energy expenditure on immune reactions. Immune function in insects is influenced by nutritional status (Feder *et al.* 1997). Several components of the insect immune system are produced by the fat body, mostly for use against bacteria and fungi (Tepass *et al.* 1994). Fat accumulation can be directly reduced by parasites, as shown in the damselfly *Calopteryx splendens xanthostoma* (Siva-Jothy & Plaistow 1999). Thus, the costs of actual resistance can be related to individual condition and, hence, the host 'quality' mediated by parasites.

In this context there are good reasons to assume that host sex matters in the ability to cope with parasite infection, for example when one sex has higher expenditure in reproduction or when the sexes differ in size (e.g. Anholt *et al.* 1991). Furthermore, using two different immuno-assays, Kurtz & Sauer (1999) recently demonstrated that female scorpion flies (*Panorpa vulgaris*) are more immuno-competent than males. Only a very few studies have focused on the differences between host sexes when para-sitism is under consideration, particularly in invertebrates (but see Agnew *et al.* 1999; Fellowes *et al.* 1999; Van Sambeek & Wiesner 1999).

Damselflies are hosts for many parasite species, frequently including ectoparasitic water mites (Corbet 1999). Using a laboratory experiment, Leung & Forbes (1997) found a strong negative correlation between survival and water mite load in pre-reproductive speci-mens of the damselfly *Enallagma ebrium*. In contrast,

Andrés & Cordero (1998) did not find an impact of water mite parasitism on host survivorship in another damselfly species when using a mark–recapture technique. Neither of these studies used experimental manipulation of the parasite load.

Water mites parasitize their hosts during the host's whole maturation period (Rolff 1997). The host's weight and size at emergence depend on larval competition, nutrition and life-history time constraints (Johansson & Rowe 1999; Plaistow & Siva-Jothy 1999). As water mites show strong engorgement within two days after emerg-ence (Åbro 1981), it can be assumed that condition at emergence is important for host survival. In addition, damselflies only start foraging when their exoskeleton has hardened, which, depending on the weather, can take between a period of hours and a few days (Corbet 1999). Damselfly females gain body mass during their matura-tion period whereas males do not (Anholt *et al.* 1991). Therefore, we expect the influence of water mites on host fitness to be greater in females than in males. In this study we had three aims: (i) to investigate whether water mite parasitism under field conditions lowers survivor-ship, (ii) to test whether the host sexes respond different-ially to parasite infestation, and (iii) to investigate whether the condition measured as weight at emergence influences survivorship.

2. MATERIAL AND METHODS

(a) *The study system*

The damselfly *Coenagrion puella* (Insecta: Odonata) is common and widespread in most parts of Europe (Askew 1988). It is a host for the ectoparasitic water mite *Arrenurus cuspidator* (Acari: Hydrachnellae) (Rolff (1997) and references within). Parasite load can be exactly determined immediately after host emergence (Rolff 1997). Water mites colonize last-instar damselfly larvae and cover a phoretic phase first (Stechmann 1978). In the study system, males and females of *C. puella* do not differ in parasite abundance at emergence (Rolff 2000). Owing to the phoretic stage it is possible to remove parasites before the onset of parasitism (Rolff 1999). When the host emerges, water mite larvae crawl from the exuvia to the newly emerged

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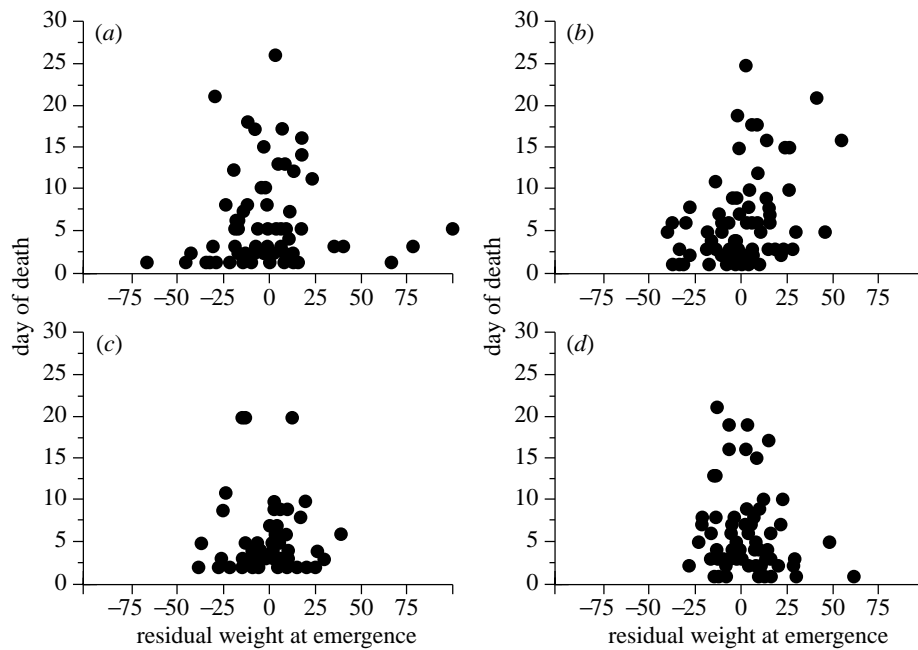
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Figure 1. Survivorship against weight at emergence of (*a*) unparasitized female, (*b*) parasitized female, (*c*) unparasitized male and (*d*) parasitized male *C. puella* in the field cages.

damselfly and then become parasites by penetrating the cuticle (Stechmann 1978). The water mite larvae feed on host haemolymph and possibly other tissues as well (Stechmann 1978). Parasitism ceases when the female host oviposits or the male host accompanies an ovipositing female. The majority of mites detach and drop into the water (Rolff 1997). Thus, parasitism acts when the host is maturing and also as their eggs develop.

(b) Study site

The pond where we caught the larvae was located in the Klei, an area with 12 small ponds at the edge of the city of Braunschweig (Lower-Saxony, Germany, 52°21′N, 10°35′E). *Coenagrion puella* emerge here in large numbers and are frequently parasitized by *A. cuspidator* (Rolff 2000). The larvae were caught during the peak of emergence in order to avoid seasonal changes in body size and parasite abundance.

(c) Manipulation of the parasite load

Manipulation of the parasite load was performed as described in detail in Rolff (1999). Damselfly larvae leaving the water for emergence were caught at the emergence substratum using a small hand net. They were stored separately in a cooling box (6 ± 3 °C) in small glass vials in order to prevent larvae from emerging and were then brought to the laboratory. All the phoretic mite larvae of the manipulated (hereafter called unparasitized) groups were removed using Dumont tweezers in a controlled temperature (CT) room (12 °C). Therefore, their natural infestation was known before the onset of parasitism. The larvae were then transferred to plastic vials with a stick serving as an emergence substrate and brought to a CT room (20 °C) where they emerged within 12 h. Their head width, weight and sex and any remaining mites were recorded after emergence. Mites in the parasitized group were counted after emergence, because they are engorged and clearly visible one day after emergence. The damselflies were marked individually

with a permanent pen on the left fore wing. They were then released into field cages.

(d) Field cages

We constructed four identical field cages (1 m × 1 m × 1.5 m) (for a detailed description see Rolff (1997)). The field cages were built in close proximity to the pond where the larvae were caught. The damselflies were fed daily *ad libitum* with Diptera caught in the wild. Damselflies were randomly distributed among the cages. The numbers of survivors were counted daily.

(e) Laboratory experiment

As field cages offer quite comfortable conditions with no predation and a good food supply, we also conducted a correlative laboratory study following the protocol of Leung & Forbes (1997). We simulated bad weather conditions by individually housing males and females caught directly after emergence in plastic jars. The air humidity was always higher than 97% and the temperature was kept constant at 10 °C. As discussed by Leung & Forbes (1997), this layout simulates the type of bad weather conditions under which damselflies are unable to forage. Here, the numbers of survivors were counted twice daily.

(f) Data analysis

We first used an ANCOVA in the cage experiment for the whole data set with sex and manipulation as factors and the residual of weight at emergence (from a regression of weight on body size in order to obtain a size-independent measure for condition (see also Siva-Jothy & Plaistow 1999) as size and weight correlate in *C. puella* (Banks & Thompson 1985, 1987)). We expected differences in the daily survival probability between adults and tenerals; therefore we expected a type-three survivorship curve, which can be analysed with a Weibull distribution (Crawley 1993). Furthermore, mites feed mainly in the

Table 1. *Results of the separate survival analyses for parasitized and unparasitized females and males when testing for the influence of body mass on survivorship*

group	n	χ^2-statistic	d.f.	p-value
parasitized females	76	13.22	1	0.0003
unparasitized females	71	0.04	1	0.8280
parasitized males	76	2.10	1	0.1470
unparasitized males	66	1.29	1	0.2660

first few days (Åbro 1981). Therefore, we analysed the parasitized and unparasitized males and females separately in order to investigate the influence of body weight.

For the laboratory data set, we first corrected weight at emergence for size as described above. We then applied correlations on the influence of mite load and weight at emergence. The ANCOVA and survival analysis were calculated using GLIM 3.77 (Royal Statistical Society, London) and all other analyses were calculated in STATview 5.0 for Macintosh (SAS Institute, Cary, NC, USA).

3. RESULTS

We followed the survivorship of 156 females and 142 males, 71 and 66 of which had manipulated parasite loads, respectively.

The parasite load before manipulation did not differ between either males and females or individuals with a manipulated parasite load and unmanipulated individuals (two-way ANOVA on square root-transformed mite abundance, sex $F_{1,293} = 0.486$ and n.s., manipulation $F_{1,293} = 0.709$ and n.s. and sex × manipulation $F_{1,293} = 0.506$ and n.s.). After manipulation they differed significantly (two-way ANOVA, manipulation $F_{1,293} = 1000.4$ and $p < 0.0001$, sex $F_{1,293} = 0.206$ and n.s. and sex × manipulation $F_{1,293} = 0.172$ and n.s.) (mean parasite load for manipulated individuals $= 2.1 \pm 2.8$ and mean parasite load for non-manipulated hosts $= 56.0 \pm 30.6$).

We used the residual body mass from a regression of body mass on head width in order to remove size effects. The full model ANCOVA (sex × manipulation × residual mass) gave a significant reduction in deviance ($F_{7,291} = 2.011$ and $p = 0.05$) with survivorship (days) as the response variable (figure 1). The interaction term (sex × manipulation × residual mass) was significant, as revealed by deletion ($F_{3,295} = 6.44$ and $p > 0.01$) Therefore, both factors, i.e. sex and manipulation and the covariate were important. The only significant two-way interaction determined by removal was sex × manipulation ($F_{2,296} = 6.66$ and $p < 0.01$). The mean survivorship ($\pm$ s.d.) was 5.63 ± 5.29 days.

The survival analysis for parasitized females revealed a significant influence of residual body mass at emergence, with heavier females having a higher survival probability. Residual body mass at emergence had no influence on survivorship in any of the other groups, i.e. unparasitized females and parasitized and unparasitized males (table 1).
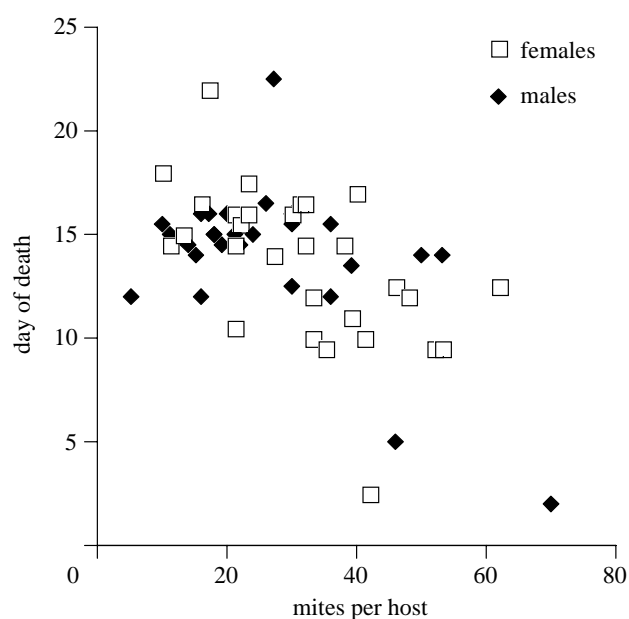
Figure 2. Survivorship of naturally infested *C. puella* in relation to parasite load in the laboratory experiment. Males and females did not differ in their responses (statistics are given in the text).

Survival in the laboratory experiment was negatively correlated with mite abundance in both males ($n = 27$, $r = -0.455$ and $p = 0.0161$) and females ($n = 29$, $r = -0.570$ and $p = 0.001$) (figure 2). Furthermore, survival was also positively correlated with residual weight in both males ($n = 27$, $r = 0.617$ and $p = 0.0005$) and females ($n = 29$, $r = 0.805$ and $p < 0.0001$). Residual weight and mite load were not correlated in males ($n = 27$, $r = 0.277$ and $p = 0.1723$) and females ($n = 29$, $r = 0.248$ and $p = 0.197$), respectively. The mean survivorship ($\pm$ s.d.) for males was 13.7 ± 3.8 days and that for females was 14.0 ± 3.7 days (ANOVA, $F_{1,54} = 0.152$ and $p = 0.698$).

4. DISCUSSION

Weight at emergence had a significant impact on survivorship in parasitized females. In contrast, weight at emergence did not influence the survivorship of parasitized males. The data demonstrated that water mite parasitism under field conditions can increase host mortality. These results were supported by the correlative results from the laboratory study. Leung & Forbes (1997) found the same mortality pattern in a correlative laboratory study using premature *E. ebrium* damselflies parasitized by *Arrenurus* water mites. However, the parasite load in the field cage experiment was manipulated, which shows that parasitism caused the increase in mortality in females rather than any trait correlated with parasite load. Andrés & Cordero (1998) did not find any difference in survivorship in a mark–recapture study of the damselfly *Ceriagrion tenellum* that is naturally infested with water mites. However, as Reinhardt (1996) showed, the flight ability of parasitized damselflies can be altered. This could result in different dispersal probabilities and, hence, confound a mark–recapture study. Forbes & Baker (1991) also found significantly lower survivorship in the mature male *E. ebrium* when they were highly parasitized (but

these data are difficult to interpret because of the way they estimated mite infection) (cf. Rolff 1997).

As the experiments were performed in a field cage it is likely that we measured the direct cost of parasitism rather than a combination of direct and indirect effects. Assuming that parasitized individuals have to obtain more food, which is justified since water mites drain nutrients and may weigh up to 20% of the body weight of their hosts (Åbro 1981; J. Rolff, unpublished data), they have to forage more. However, foraging is costly in terms of predation risk (Rehfeldt 1992). Therefore, the impact of parasitism on survivorship was presumably under-estimated in the field cages. In addition, food was available *ad libitum* within the cages.

The laboratory study exerted a severe impact on the damselflies as they were unable to feed and forage at all and, therefore, sexual differences in behaviour and weight gain during the pre-reproductive phases (see Anholt *et al.* 1991, and discussion below) were prevented. The longer survival compared with the field cages was due to the low temperature (10 °C). Even though both sexes showed a significant decrease in survivorship that was correlated with mite load, this disappeared in males if the point at the bottom right of figure 2 was removed. This suggests that, in females, the impact of parasites is stronger than in males and indicates that the effect of parasitism and the different responses of the sexes are strongly dependent on the environmental conditions.

Weight at emergence is determined by food uptake in the last larval instar. Three aspects are important: intra-guild predation, food supply and the timing of emergence. Depending on the level of competition the size of the larvae changes (Johansson 1996). The food supply also determines size and weight at emergence (Plaistow & Siva-Jothy 1999). Furthermore, the timing of emergence is determined by evolutionary forces such as the time of the season and the availability of mating partners (Johansson & Rowe 1999). More proximately, the weather conditions and individual flexability to respond to unfavourable conditions for emergence, males being more flexible than females (Baker *et al.* 1992), influence the timing of emergence and, hence, individual condition. This is the ecological context in which water mite parasitism is embedded. It should be further noted that condition did not correlate with mite load, underlining the importance of condition and parasitism in the survival of *C. puella*.

Females of coenagrionid damselflies gain weight during maturation whereas males do not usually gain weight (Anholt *et al.* 1991). The reason for this is that females need more reserves for producing eggs. Their first reaction to parasitism is to reduce egg numbers (Rolff 1999). Females probably have to trade off survival against reproduction under nutritional deprivation. This costly trade-off might explain why females starting with lower weights are more affected by parasites. Siva-Jothy & Plaistow (1999) showed that calopterygid damselflies parasitized by gregarines during the teneral stage show reduced fat accumulation. As far as we are aware, only Van Sambeek & Wiesner (1999) and Agnew *et al.* (1999) have shown differences in survival between host sexes, in a locust and a mosquito, respectively. In addition, Léonard *et al.* (1999) provided data on sexual differences in survival in a damselfly. However, they superinfested damselflies para-sitized by *Arrenurus* mites with *Limnochares* mites and looked for the effect of the latter ones. This makes a comparison difficult as nothing was reported about the survival effects of the primary infection.

Whereas a great deal of the difference in parasite influence on the host sexes in vertebrates might be due to the hormone testosterone (Folstad & Karter 1992), other factors such as reproductive effort are probably of more importance in invertebrates as they lack such sex-specific hormones (Nijhout 1994). *Coenagrion puella* females have high costs of reproduction and their lifetime reproductive success is determined by longevity (Banks & Thompson 1987). In contrast, males can maximize their lifetime mating success by either increasing their daily mating rate or longevity (Banks & Thompson 1985). *Coenagrion puella* males, like many other dragonfly species' males, 'scramble–compete' and, therefore, do not invest in orna-mentation.

As recently shown by Kurtz & Sauer (1999) in the scor-pion fly *P. vulgaris*, the immunocompetence of male and female insects can differ as well. They found that females were more immunocompetent, as measured by lysozyme activity and phagocytosis, than males. However, such differences were not as clear in the damselfy *Lestes viridis* (Rolff, unpublished data). Therefore, we suggest that the underlying mechanism for the sexual differences in para-site virulence as reported here is the difference in repro-ductive investment as outlined above. Furthermore, in the laboratory experiment, where the damselfies were hindered from displaying their normal behaviour, sexual differences could not be detected.

Theory predicts different consequences of parasitism on host population dynamics depending on the host traits that are affected by the parasitism (May & Anderson 1978). As shown here and in related studies (Rolff 1999; Rolff *et al.* 2000), several alterations can occur in one particular host–parasite association and their intensity depends on the ecological factors altering or constraining a host's life history. In addition, the host sexes can also respond differently to parasitic infestation, as found in the survivorship of damselflies.

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