FITNESS-RELATED ATTRIBUTES AND GREGARINE BURDEN IN A NON-TERRITORIAL DAMSELFLY *ENALLAGMA PRAEVARUM* HAGEN (ZYGOPTERA: COENAGRIONIDAE)

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Odon. are usually infected with intestinal gregarines. Using *E. praevarum* adults, it was investigated whether: (a) both sexes differed in the degree of parasitism and immune ability (as shown by melanization of artificial, nylon-based implants in the thoracic region); and, (b) gamete production, survival and fat reserves correlated with gregarine burden. 2 sets of in-copula (to control for age) animals were used. One was used for estimation of egg and sperm, and the other for fat reserves. Survival was monitored as the time that field-captured insects survived under laboratory conditions in the absence of food. Gregarines were counted by dissection of the gut. Despite the case that ♀♀ had more parasites than ♂♂, both sexes did not differ in immune ability. Eggs, but neither sperm nor fat reserves in both sexes, correlated negatively with parasite number. Survival in both sexes also correlated inversely with gregarine burden. This, however, held only for ♂♂ when the analysis was performed by sex. These results are discussed in terms of the detrimental effects of gregarine on Zygoptera hosts.

INTRODUCTION

Recent studies in evolutionary biology have focussed on the pervasive action of pathogens on different animal species. In particular, studies in some odonate species have found detrimental effects of gregarines on different fitness related traits in both sexes (e.g. ÅBRO, 1974, 1976; SIVA-JOTHY & PLAISTOW, 1999; CÓR-

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DOBA-AGUILAR, 2002; HECKER et al., 2002; CÓRDOBA-AGUILAR et al., 2003). Gregarines are protozoan, mostly insect parasites whose life cycle takes two sequential routes: a noninfective (having several insects as vectors) and an infective stage (within the host's intestine tract) (ÅBRO, 1976). Gregarines have a negative effect on adult survival (e.g. CÓRDOBA-AGUILAR, 2002; CÓRDOBA-AGUILAR et al., 2003), egg production (CÓRDOBA-AGUILAR et al., 2003), fat reserves in flying muscles (SIVA-JOTHY & PLAISTOW, 1999) and female mating decisions (SIVA-JOTHY, 2000; CORDOBA-AGUILAR et al., 2002).

Hosts are not passive actors in the interaction with parasites. Insects develop an immune response against parasites, which is based on a melanin-based encapsulation of the foreign agent (LAVINE & STRAND, 2002). This response effectively isolates the invading agent, preventing its development and killing it eventually. The use of melanine possibly affects other insect's structures as this compound is also necessary for other functions, one example being the elaboration of male sexual traits aimed to increase the individual's potential to attract mates (e.g. SIVA-JOTHY, 1999, 2000; HOOPER et al., 1999). The host, therefore, faces the physiological dilemma of how much melanine is diverted to immune response and how much to other functions. This sort of tradeoff has in fact become a cornerstone in the evolutionary thinking of the parasite-host interaction (ROLFF & SIVA-JOTHY, 2003).

In theory, the sexes differ in their immune ability. As females increase their fitness by augmenting their longevity (to produce more eggs) while males maximize their mating success, females should invest more in immunity than males (ROLFF, 2002). This prediction has been confirmed in the Japanese beetle, *Popillia japonica*, in which females exhibit higher levels of immunity than males (TUCKER & STEVENS, 2003). To what extent this applies to other animal forms awaits investigation.

Enallagma praevarum is a small damselfly. The males gather in lentic waters, occupying the plant substrates that females use for oviposition. *E. praevarum* males do not appear to defend territories, and they mate with any females that arrive at oviposition sites. Similar to other odonate species, a considerable number of individuals are parasitised by gregarines, but no study has shown the effects of this parasitism. In this paper we document the possible negative impact that gregarines bear on male and female adults of *E. praevarum* examining three fitness attributes: gamete production (eggs and sperm), survival, and fat reserves. Furthermore, we also investigated whether there was a sexual difference in immune response.

MATERIAL AND METHODS

E. praevarum adults were collected in the Venados River in the Metztitlán reserve, Hidalgo, Mexico in summers of 2002 and 2003. For differences in parasitism between the sexes, 60 males and 69 females of middle age (animals with shiny body colour, transparent wings and no pruinescence) were used.

For sexual differences in immune response, a sample of 69 animals (61 ♂, 8 ♀) was randomly collected. A 1.5 mm long and 0.1 mm wide plastic nylon implant was inserted in the dorsal side of the tho-

rax of each animal. Previous to insertion, the implant was disinfected by immersion in ethanol (100%) for 2 seconds. The damselflies were individually maintained in small vials during 28 h with L/D cycles of 12 h. In using the nylon implant, the insect immune system proceeds recognizing it as a foreign, potentially infective agent, covering it with several haemolymph cell layers which get melanized after some hours; this melanization is a way to isolate and kill the invader (LAVINE & STRAND, 2002). Given that immune response in damselflies is usually completed after 24 h (SIVA-JOTHY et al., 2001), 28 h seems the best logistic compromise for this protocol. Animals were hand fed using one *Drosophila* adult per damselfly after 12 h of maintenance. The implant was then removed by gently dissecting the area where it was inserted by using fine forceps and transferred to ethanol. After 2 h of re-hydration, the implants along with the melanization were photographed. A relative value of the melanization area (which, due to its darkish nature, appears totally visible on the transparent nylon implant) was measured with image analysis software.

For gamete production, 46 and 127 in-copula females and males, respectively, were collected and preserved in ethanol (70 %; the same concentration was used for the other animals described below). These individuals were later dissected and the eggs counted under a stereoscope microscope. The in-copula condition provided some reliability that these animals had reached sexual maturity and thus had produced gametes. Males were collected and maintained under laboratory conditions (24 °C and L/D cycles of 12 h) during 24 h to allow for sperm production in case they had engaged in copulation recently and had, therefore, become sperm exhausted. After this time, these males were preserved also in ethanol and then re-hydrated for 24 h. Sperm masses from these animals were dissected from the sperm vesicle and primary gonopore. These masses were laid on a slide, squashed with one coverslip held over two other coverslips at either side (all coverslips of 1 mm wide) and video captured. The sperm mass areas displaced were calculated on image analysis software.

For survival, 117 (103 ♂, 14 ♀) animals were collected, taken to an insectary (1 m × 1m × 1 m), and left deprived of food. Every two hours, the number of surviving and dead animals was counted. In every checking, dead animals were immediately preserved. Counting of gregarines was made via dissection of the gut tract after re-hydration of animals for 24 hours.

For fat calculation, 49 pairs were captured. Controlling age in damselflies for fat calculation is important, as relatively young and old animals may be fat depleted due to initial construction of fat reserves in the former and fat depletion due to sexual activities in the latter (e. g. PLAISTOW & SIVA-JOTHY, 1996). For testing the effect of parasites on fat reserves, therefore, middle aged animals should be available. We assumed that our animals were of this age for the following reasons: (a) they were sexually active (so that they were not young - at least not in that stage at which fat accumulation is the animal's main function – and not old – otherwise they would not copulate); and, (b) the body colour (an indicator of age in these animals, CORBET, 1999) was similar among individuals These animals were immediately decapitated; the last two abdominal segments were pulled off to allow for intestine parasite extraction and preserved. The rest of the body was left in glassine bags for 24 h in a desiccator, at which time body weight was recorded. These structures were left again in chloroform for drying in a desiccator for 24 h, at which time another weight recording was obtained. The absolute difference between weights was taken as the fat quantity the animal had previous to chloroform extraction (see also PLAISTOW & SIVA-JOTHY, 1996).

Sperm masses and melanization areas were quantified using Sigma Scan Pro 5.6 ® image analysis software. When possible, parasite data were transformed as (root square (value + 0.5)) for their analysis using parametrical tests. Results are shown as mean ± STD unless otherwise stated.

RESULTS

There was a difference in the number of parasites: females (3.05 ± 4.44, N = 69) were more heavily parasitized than males (2.33 ± 4.45, N = 60; t = 2.12, P =
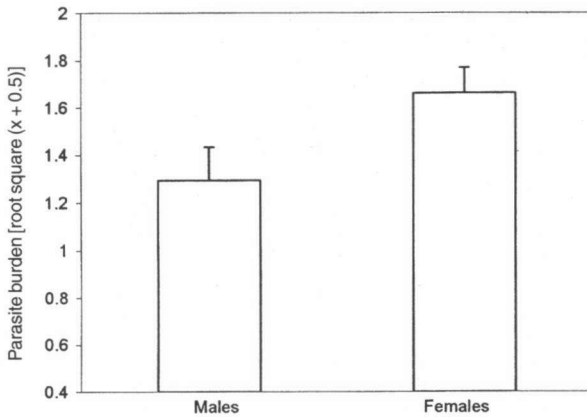
Fig. 1. Sexual differences in gregarine burden in *E. praevarum*

0.03, d. f. = 127; Fig. 1). However, the sexes did not differ in their degree of melanization (males, 0.05 ± 0.03, N = 61; females, 0.04 ± 0.04, N = 8; t = 0.69, P > 0.05).

There was a negative relationship between egg number and parasite burden (r = - 0.33, P < 0.01, N = 46; Fig. 2). Sperm mass, however, was not related to parasite number (r = - 0.006, P > 0.05).

The sexes did not differ in survival (survival for males = 19.89 ± 8.11, N = 103; for females = 18.42 ± 4.58, N = 14; Mann Whitney U test = 0.71, P > 0.05). Survival was related to parasite number (r = -0.32, P < 0.001, N = 117): more heavily parasitized animals were less likely to survive. This result held for males (r = -0.33, P < 0.0006; N = 103; Fig. 3) but not for females (r = 0.38, P > 0.05, N = 14), when sexes were analysed separately.

Gregarine number was not associated to fat reserves in males (r = 0.16, P > 0.05, N = 23) or females (r = 0.02, P > 0.05, N = 24), and this result still held when animals with no parasites were eliminated (males, r = -0.14, N = 11; females, r = -0.13, N = 20; both P > 0.05). Even when comparing parasitised (N = 31) and non-parasitised (N = 18) animals, these results did not change (parasitized 5.17 ± 3.69, non parasitised 4.48 ± 3.18; t = 0.66, P > 0.05).
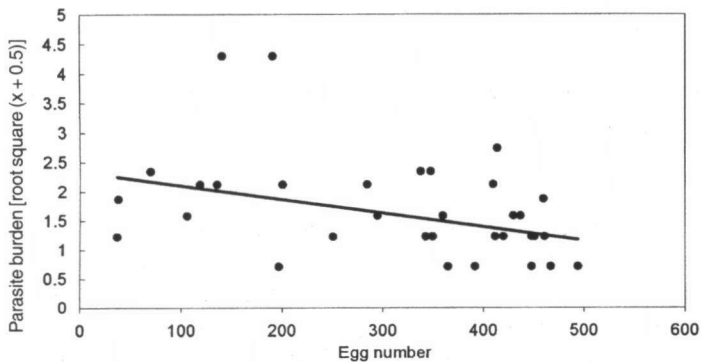

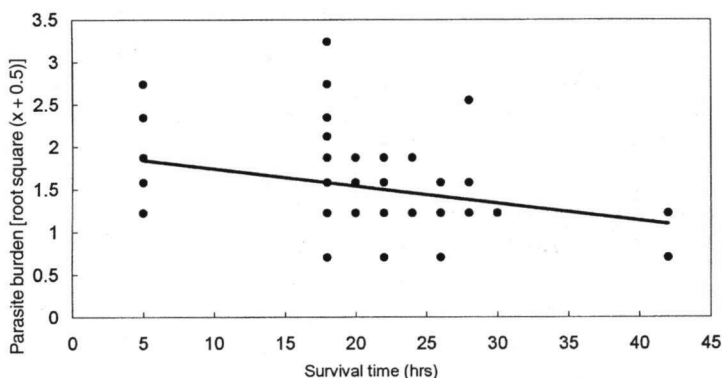Fig. 2. Parasite burden in relation to egg production in *E. praevarum*

Fig. 3. Parasite burden in relation to survival in *E. praevarum*

DISCUSSION

Recent estimates of the evolutionary effects of gregarines in damselflies have shown a set of fitness-related surrogates that are detrimentally affected. We have explored some of these surrogates which, in different extents, have been also investigated in other species.

Immature is the adult period during which the animal's main activity is feeding. This time is also crucial as the animal starts being infected (ÅBRO, 1974, 1976). While the animal is maturing, it is becoming more parasitized. If both sexes share the same feeding habits (e.g. foraging in the same places, capturing and ingesting prey at the same rate) and immune abilities, similar intensity of parasite infection may be found. This is, surprisingly and unlike other studies in damselflies (e.g. HECKER et al., 2002) and arthropods in general (SHERIDAN et al., 2000), not the case for *Enallagma praevarum*. The explanations why females have more parasites than males possibly are that females feed in places where gregarines are more common or that females feed at a higher rate than males so that they become more frequently infected. This is, however, inconsistent with the fact that both sexes apparently have similar immune abilities, shown by the nylon melanization, another result which contradicts what is theoretically (ROLFF, 2002) and pragmatically (e.g. KURTZ et al., 2000; KURTZ & SAUER, 2001; YOURTH et al., 2002) expected. One possibility is that immune response had not been completed by the time the implant was extracted. However, even if this were the case, still some differences should have become apparent. At present, there are no explanations for these divergences.

The fact that gregarines feed on the damselflies' ingested food possibly signifies a reduction in the energetic value of the food gathered by the adult. This may be costly, particularly for females, as the energetic demand exerted for egg production can be especially high. In line with this, our results suggest that females have reduced egg numbers when parasitism incidence is high. Similar results have been found in

the territorial damselfly *Calopteryx haemorrhoidalis* (CORDOBA-AGUILAR et al., 2003). This impact may have further costs for females if the number of eggs carried by a female can be detected by a male. In *C. haemorrhoidalis*, in fact, it has been suggested that males devote less time for post-copula guarding when females have few eggs to lay (CÓRDOBA-AGUILAR et al., 2003). Apparently, males may "evaluate" females' weight and hence adjust how long they stay with them. These observations should also be carried out in *E. praevarum*.

The cost of parasitism observed in the number of eggs may not necessarily be the consequence of food competition between the parasite and the host. The reduction in egg production may also come as a product of the immune response that the host is producing against parasites. For example, it may be that a compromise emerges in using melanine, the basis of the cellular immune response (LAVINE & STRAND, 2002), for dealing with parasites and not for other functions in which this component is also vital (e.g. SIVA-JOTHY, 2000). In mosquitoes, for example, melanine is the main component in the egg chorion (JOHNSON et al., 2001). Given this one may easily translate the compromise of melanine destined for egg production and not immunity. Such a trade off is an interesting pathway worth of study.

The negative relationship between egg number and parasite burden contrasts with that of males in *E. praevarum*, in which sperm production was not apparently affected. Possibly, the fact that eggs seem more energetically costly to produce leaves females with more difficult physiological imbalances when faced with a higher parasite burden. A similar relationship was observed for fat reserves in both sexes. This is paradoxical, given that parasites have been shown to directly affect lipid-based compounds from invertebrate hosts (FOLLY et al., 2003). In fact, this is exactly the cost that damselfly adults would suffer from parasites of the life mode such as that of gregarines (e.g. SIVA-JOTHY & PLAISTOW, 1999; for another example with ectoparasites see ROLFF et al., 2000). It would be expected that the main direct effect is on fat acquisition from ingested food. A set of explanations can be put forward for our negative results. First, although we tried to control for age differences, some animals were possibly too old and hence already fat-depleted. It has been shown that relatively old damselfly males are fat exhausted most likely due to highly costly sexual activities (PLAISTOW & SIVA-JOTHY, 1996). The fact, however, that the adults used were copulating clearly suggests that most, if not all, animals were far from being fat depleted. Second, it may be that the fat effect due to parasites had already taken place when the animal was captured, but given that damselflies get infected continuously, the current parasite burden would not have reflected fat depletion. This is not true, as a number of collected damselflies were not infected, and the comparison of these individuals with infected damselflies still did not produce any difference.

Survival was negatively correlated with gregarine number. This result does not parallel previous findings in *Enallagma boreale*, whose adults survived for longer periods when parasitism was higher (HECKER et al., 2002). Presumably, in the ab-

sence of food, animals should become more stressed and hence the effects of parasite start be clearer. Despite experiencing the same conditions, our results and those of Hecker and collaborators are strikingly different. Perhaps one difference is that they monitored the animals' condition every 12 hours, a time at which much mortality had already occurred. In our experience, 2 hours was the best logistic compromise to observe the occurrence of dead *E. praevarum* adults. Possibly, intervals of 12 hours masked the gradual mortality occurring within this period, Interestingly, *E. boreale* clearly survived for longer than *E. praevarum*: while the formed had surviving rates of 8.2 ± 0.3 days for females and 5.8 ± 0.4 days for males, the latter never reached a single day. Possibly this difference may reflect population-based adaptations to local changes in stress conditions. Damselflies in general are subject to weather changes that leave them with periods of no food (CORBET, 1999). *E. boreale* from Canada may be well adapted to situations like this in which the animal may have to tolerate several days of bad weather. For *E. praevarum* from central Mexico, inclement periods of bad weather may be of shorter duration, and therefore damselflies may be subject to less prolonged intervals without food.

Interestingly, females did not show a diminished survival compared to males in relation to gregarine burdens. This result can be interpreted on the basis of the priorities in energy allocation that both sexes have evolved (ROLFF, 2002). According to this, males should invest in activities that directly maximize their reproductive success, while females should invest in egg production. In males of nonterritorial damselflies, an important component of lifetime reproductive success is longevity (CORBET, 1999). This might be the case in *E. praevarum* males in which the high energetic demand devoted to survival of which may be heavily affected by parasites. In females, this should not necessarily be the case, as possibly survival may not be a direct target of selection while egg production is.

In summary, our correlates show only that egg production and male survival seem affected by gregarines, which is in agreement with what it is predicted by evolutionary ecology theory (ROLFF, 2002). Furthermore, females seemed more heavily parasitised than males, a result that is apparently contradicted by the similar immune responses shown by both sexes. Given that these controversial results are not unique for nonterritorial damselflies (e.g. YOURTH et al., 2002, HECKER et al., 2002), the scene is set for further research.

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