

A fitness cost of eugregarine parasitism in a damselfly

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Abstract. 1. Adult *Calopteryx splendens xanthostoma* are infected by the trophozoites of a eugregarine parasite that attaches to the mid-gut epithelium.

2. The results of this study show: (1) that eugregarines were the predominant parasite in the isolated study population, (2) they were not present in imagos that had just eclosed from larvae but were present in significantly higher numbers in older adults, (3) animals with a eugregarine burden during the prereproductive fat-accumulation period of their adult development (the teneral stage) had significantly lower fat content, and (4) males infected artificially with eugregarine oocysts showed no correlation between eugregarine burdens and postmanipulation fat content.

3. Infection with eugregarine trophozoites during the teneral life-history stage affects the ability of damselflies to accumulate fat, and consequently reduces their ability to fight for, and maintain, a territory when they become reproductively active.

Introduction

Parasites are important in driving the evolutionary and population dynamics of many host organisms (e.g. Anderson & May, 1978, 1991). Theory predicts that they determine the qualitative and quantitative nature of components of male secondary sexual traits (e.g. Hamilton & Zuk, 1982) and they increasingly affect the way in which evolutionary ecologists view the interaction between life-history phenomena and ecological patterns (e.g. Sheldon & Verhulst, 1996). Pivotal in understanding the many perceived roles that parasites play in evolutionary ecology are the costs they impose on hosts. These costs form a continuum, marked at one end by the costs paid by a host for maintaining complete resistance, and at the other by the costs of maintaining a parasite burden while investing minimally in immunity.

Most parasites have life-history strategies that cause morphological and/or physiological damage to hosts, but quantifying the fitness costs of such damage (i.e. the cost of maintaining a parasite burden) is often difficult because the scale of host costs is determined by the virulence of the parasite (i.e. how easily it can breach the host's defences) and the damage it causes. It is important to bear in mind that parasites do not always have measurable effects on host fitness (e.g. Goater & Holmes, 1997) but several studies have now

documented sublethal fitness effects of parasites in vertebrate hosts (e.g. Møller, 1990; Connors & Nickol, 1991). The aim of the study reported here was to assess the effect of infection with eugregarine trophozoites on the fat content of male *Calopteryx splendens xanthostoma* prior to entering the energetically demanding contests that enable them to acquire and maintain a territory. Territory occupancy is a major determinant of fitness (Plaistow & Siva-Jothy, 1996).

Gregarines are extracellular sporozoan (Protozoa) parasites of invertebrates (Smyth, 1976). Within the family Actinocephalidae, the eugregarines are cosmopolitan mid-gut parasites of damselflies (Zygoptera; Odonata) (Åbro, 1971, 1990, 1996) and appear to rely largely on adult hosts for transmission (Åbro, 1971, 1976), although there are reports of infected larvae (Schneider, 1875; Geus, 1969). Infection occurs via the ingestion of oocysts attached to the legs of the small flies on which the damselflies feed (Åbro, 1976). The oocysts germinate and release the infective sporozoites, which then attach to the host's mid-gut epithelium and develop into trophozoites. The trophozoites develop in the gut, and eventually fuse before being expelled from the host as a gametocyst, which contains thousands of infective oocysts. Adult hosts therefore ingest oocysts and loose mature parasites as part of the natural cycle of infection. Hosts that have high burdens of trophozoites often have ruptured and/or blocked mid-guts, which can result in reduced longevity (Åbro, 1990). Longevity is a major determinant of fitness in some damselflies (Banks & Thompson, 1985). Calopterygid damselflies are resource defence polygynists, however, so longevity is

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probably less important in determining male fitness than the ability to obtain and defend a territory. Because reproductive success is 1000 times greater for territorial males of the damselfly *C. s. xanthostoma* than for males that adopt the alternative mate-securing tactic (Plaistow & Siva-Jothy, 1996; Plaistow, 1997a), it follows that the duration for which an individual can hold a territory will underpin male fitness in this species. Studies of calopterygid damselflies have shown that fat reserves accumulated during the prereproductive adult *teneral* phase determine the ability of a male to engage in sustained flight (odonates burn fat in their flight muscles; Kallapur & George, 1973), which in turn determines whether a male can secure and maintain a territory (Marden & Waage, 1990; Marden & Rollins, 1994; Plaistow & Siva-Jothy, 1996).

A potential cost of any digestive-tract parasite may be a reduction in the ability of an infected host to absorb nutrients and therefore accumulate fat reserves. Damselflies are subject to infection by other parasites, in particular microsporidians (e.g. Weiser, 1961, 1963), trematodes (e.g. Boddeke, 1960a; 1960b), and ectoparasitic mites (e.g. Rolff & Martens, 1997). All of these parasites might also affect the ability of individuals to accumulate fitness-enhancing resources. The potentially confounding effects of multiple infective agents need to be considered in any study of parasite effects on hosts.

This paper reports the results of field-based observational study and an experiment designed to identify the potential cost (in terms of reduced fat accumulation) of eugregarine parasitism in the males of the sexually dimorphic damselfly *C. s. xanthostoma*. First, the study population was examined to identify the prevalent parasites. The frequency distribution of the prevalent parasite was then evaluated across different age categories of adults (an indication of the parasite's infection strategy). Parasite infection during the *teneral* stage was predicted to be associated with a reduction in the fat reserves of infected males prior to the onset of energy-demanding reproductive activity. If variation in larval feeding results in variation in *teneral* fat loads, or if fat confers an advantage in resisting eugregarine infection (the fat body is responsible for secreting humoral immune effector compounds in insects: e.g. Hetru *et al.*, 1998), there should be a relationship between parasite burdens and fat reserves similar to that predicted above. This causal possibility was examined by testing experimentally the prediction that higher fat reserves confer resistance to eugregarine infection.

Materials and methods

Study site

This study was conducted on a 2-km stretch of the Vidourle River in southern France (43°52'N, 04°03'E) in 1997 and 1998.

Observations and collection of males

All reproductively active males at the isolated study site were marked individually with acrylic paint spots on the thorax

and abdomen. All males were aged using the criteria outlined below, then either marked and released, or kept for analysis of trophozoite burdens. Preterritorial males ($n=12$) (adult males that had just finished their *teneral* phase and were beginning reproductive activity: Plaistow, 1997a) were caught between 10.00 and 18.00 hours (between 9 and 30 July 1998) as they appeared for the first time in the population of individually marked insects. Although preterritorial males are relatively rare in the population, they are the only life-history stage in which fat loads can be interpreted meaningfully in relation to parasite burdens because these males have just finished accumulating their fat stores (Plaistow, 1997b; Plaistow & Siva-Jothy, 1999). The relatively small sample size in this study reflects the difficulty of capturing these males prior to reproductive activity, but is offset by the low levels of *noise* in the data set.

All males that were captured and used for fat analysis and/or trophozoite counts were stored in a cool, dark box for no more than 4 h after capture. They were then decapitated and gutted before being stored in paper envelopes in a sealed box containing silica gel. This storage process dried out the soft body tissues rapidly and was ideal for preparing and storing samples for subsequent fat extraction. If trophozoites were being counted, the gut was dissected immediately (see below).

Freshly eclosed males were also collected throughout the season whenever they were found. These insects were identified easily because their cuticle was still soft and their wings were glassy in appearance. These males were dissected to evaluate trophozoite burdens carried over from the larval stage.

Sample preparation for light and electron microscopy

Samples for light microscopy were fixed in Bouin's fixative (Bancroft & Stevens, 1990) in the field and subsequently embedded in wax prior to cutting 8- μ m transverse sections of the abdomen that were stained with haematoxylin and eosin. Samples for transmission electron microscopy were fixed in 2% glutaraldehyde solution in cacodylate buffer (pH 7.3, with 7% sucrose), washed and post-fixed in 1% osmium tetroxide before being embedded in CY212 resin prior to cutting thin sections (80 nm). All sections were stained with lead citrate and counter stained with uranyl acetate prior to examination in a Philips EM400 at 80 kV [See Bancroft & Stevens (1990) for details of both techniques.]

Evaluating eugregarine burdens

The number of eugregarine trophozoites in a male's mid-gut was assessed by removing the entire gut carefully and placing it into a dissection dish containing ice-cold hypotonic saline solution (90 mM NaCl). The transparent mid-gut epithelium was pierced and dissected with a narrow gauge (21 G) hypodermic needle. The resultant sheet of epithelium was then pinned out in the dissecting dish. After 30–60 s, the

trophozoites swelled up in the hyposmotic saline and stood perpendicular to the epithelial sheet, facilitating counting.

Fat extractions

Fat extractions were carried out on the thorax and abdomen using the protocol of Plaistow and Siva-Jothy (1996). Body parts were reweighed after fat extraction to obtain the dry fatless mass. Fat content was calculated from the difference between the dry mass and the dry fatless mass. This value is not presented as the residual from a regression of total fat content over dry fatless body mass (see Marden & Rollins, 1994) because the relationship was not significant in the data set.

Age categories

Absolute age could not be determined by the date of marking because observations did not begin at the start of the season, and the youngest imagos feed away from the stream so they are difficult to observe and mark (e.g. Forsyth & Montgomerie, 1987). Males were therefore aged by assigning them to one of five age categories (see also Marden & Waage, 1990; Siva-Jothy & Tsubaki, 1994) defined mainly by the stiffness of the leading edge of the wings, which increases with age. Emergent males were always found early in the morning near the larval exuvium from which they had emerged: they had the characteristic soft translucent cuticle of a recently eclosed insect. Teneral males had brilliant metallic blue colouration while all other males had brilliant metallic blue cuticle. Males in age class 1 had undamaged wings that were dorso-ventrally flexible along their whole length. Age class 2 males had harder wings that were only flexible from the nodus to the tip. Age class 3 males had hard wings that were flexible only at the distal tip and showed slight wear.

Manipulating trophozoite burdens

Fresh eugregarine gametocysts were collected from the faeces of captive male and female damselflies and stored in the shade at ambient temperature and humidity for 7 days. They were then disrupted in stream water at a dilution of two gametocysts per millilitre of stream water: this solution was never kept for longer than 24 h. A vial of unmanipulated stream water (collected at the same time as the spore-inoculated solution) was used as a control. All reproductively active males in age category 2 were then collected from an isolated site within the study area and allocated at random to one of two treatments. Treatment males were fed 2 µl of vortexed oocyst inoculated water and were marked individually and released. Control males were fed 2 µl of uninoculated stream water under the same conditions. After release, all males soon resumed their precapture activities. After 10 days, all the treatment and control males that were still present were collected; these

were stored and processed as outlined above. This experiment was conducted in 1997 and 1998.

Results

The prevalent parasite

Eugregarine trophozoites were commonly found in the mid-gut of adult male and female damselflies throughout the reproductive season at the isolated study population.

Ectoparasitic mites have never been encountered in 10 years of observations based on the handling and marking of individuals ($n = 3857$); moreover, light microscope thin-section preparations ($n = 36$ individuals), transmission electron microscope preparations ($n = 9$ individuals), and dissections of fresh and prepared material ($n = 1023$ individuals) have failed to reveal the presence of microsporidian spores, sporogonial plasmodia, or trematode metacercariae.

Trophozoite infection levels and male age

Freshly eclosed males ($n = 22$) never contained trophozoites (Fig. 1), suggesting that larval infection is, at most, rare. By contrast, males caught during the teneral stage (the prereproductive adult phase) and in subsequent age categories contained higher trophozoite burdens: the mean trophozoite burden differed across these different age categories (Kruskal–Wallis, $H = 13.1$, $d.f. = 4$, $P = 0.01$; Fig. 1). The percentage infection rate in each age class was: eclosed males 0% ($n = 22$), teneral males 37% ($n = 27$), age class 1 males 30.3% ($n = 33$), age class 2 males 36.1% ($n = 118$), age class 3 males 50% ($n = 22$).

Trophozoite burdens and preterritorial male fat content

There was a significant negative relationship between the number of gregarine trophozoites attached to the mid-gut epithelium of prereproductive males and the residual fat content of those males prior to reproductive activity (Pearson's correlation, $r^2 = 0.49$, $d.f. = 11$, $P = 0.01$; Fig. 2).

Does fat content affect infection rate?

Because year had no effect on parasite burdens (ANOVA, $F = 0.8$, $d.f. = 1,36$, $P = \text{NS}$) and there was no interaction between year and the significant effect of the experimental treatment (ANOVA interaction, $d.f. = 1,36$, $F = 0.17$, $P = \text{NS}$; experimental treatment, $F = 19.9$, $d.f. = 1,36$, $P < 0.001$), the treatment and control data from each year were pooled.

Artificial infection with eugregarine oocysts produced a significant effect on trophozoite burdens (3.62 ± 0.81 trophozoites male^{-1} , $n = 21$) when compared with the control (0.42 ± 0.18 trophozoites male^{-1} , $n = 19$) and unmanipulated males in age category 2 (i.e. the age category 2 males in Fig. 1)

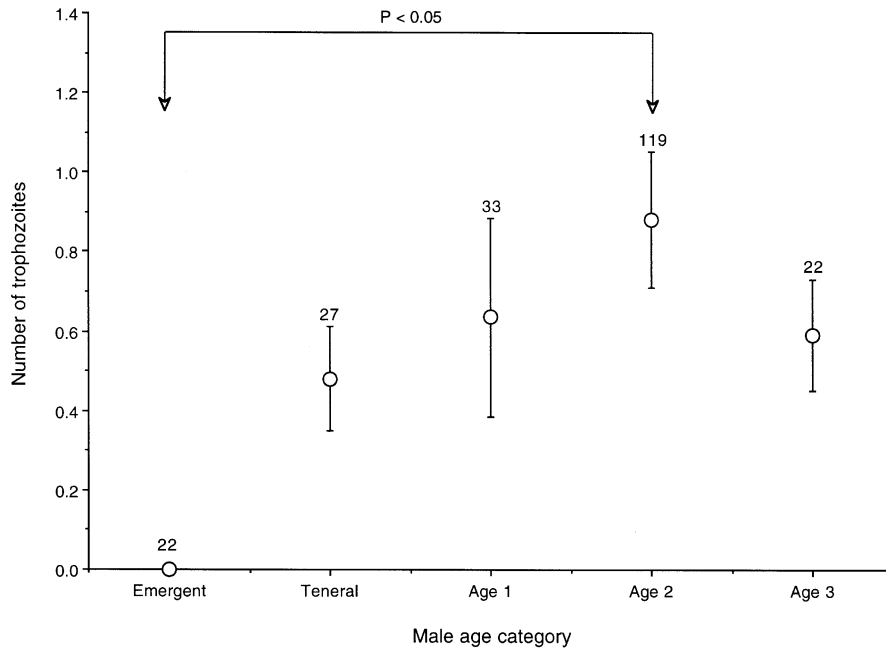


Fig. 1. The trophozoite burdens (mean \pm SD) of adult males in different age categories. Emergent adults had no trophozoites, suggesting that infection in the larval stage is very low at most. Pairwise comparisons between means (Siegel & Castellan, 1988) revealed that the only significant difference in trophozoite burdens ($P < 0.05$) was between emergent males and males in age category 2.

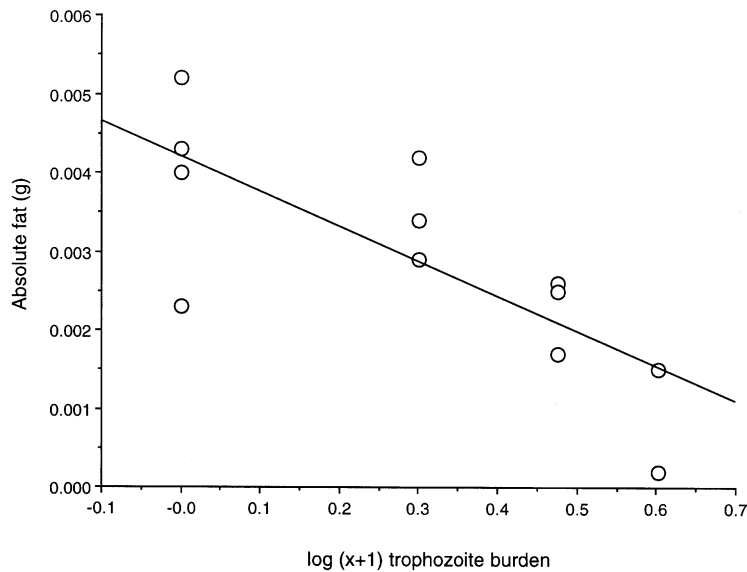


Fig. 2. The relationship between the absolute fat reserves of prereproductive adult males and their trophozoite burdens.

(0.88 ± 0.17 trophozoites male⁻¹, $n = 119$) (ANOVA, $F = 17.7$, d.f. = 2,156, $P < 0.001$). The manipulation resulted in higher burdens than in the control (Bonferroni/Dunn, $P < 0.001$) or unmanipulated age 2 males (Bonferroni/Dunn, $P < 0.001$).

There was no difference in trophozoite burdens between the control or unmanipulated age 2 males (Bonferroni/Dunn, $P = \text{NS}$). Manipulation was therefore manifest as a measurable increase in trophozoites resulting in higher than average

burdens compared with those that occurred naturally. If fat loads affected gregarine resistances there should be a negative relationship between fat load and parasite burden in the data set resulting from the manipulation.

There was no relationship between the fat content of manipulated males and their manipulated trophozoite burdens (Pearson's correlation, $r^2 = 0.001$, d.f. = 20, $P = \text{NS}$) or between the fat content of control males and their natural trophozoite burdens (Pearson's correlation, $r^2 = 0.001$, d.f. = 18, $P = \text{NS}$). A male's fat content does not appear to confer a resistance advantage against eugregarines under the conditions of this experiment.

Discussion

The presence of eugregarine trophozoites in the mid-gut of preterritorial males was correlated negatively with their fat content. It is likely that eugregarines are causal in this relationship but there are other possibilities. The relationship could be caused by infection by other parasites that were not measured, pre-adult infection with eugregarine trophozoites, or variation in the size of the teneral fat body that resulted in correlated variation in eugregarine resistance.

The results suggest that none of the common damselfly parasites was present in the study system. It is possible that these parasites exist at low levels and/or there are other, unidentified microparasites; however, the results show that the predominant parasite is a eugregarine. It is therefore unlikely that infection by unmeasured (but correlated) parasite burdens is responsible for the negative correlation between eugregarine trophozoites and prereproductive male fat reserves.

The data on trophozoite levels in successive age categories of males indicate that larval infection is not measurable (and therefore, at best, relatively low) compared to adult infection in this study. Moreover, the data suggest that adults continue to acquire eugregarines during adulthood, because there was a significant pairwise difference between emergent males and males in age category 2. The nonsignificant fall in parasite burdens in age category 3 males could be due to either decreased exposure coupled with loss of trophozoites as they fuse and are expelled as gametocysts, higher levels of resistance in earlier emerging males (which go on to become unburdened older males), or higher survival in males with smaller eugregarine burdens.

There are conflicting reports on the role of host larvae in the life history of the eugregarines that infect damselflies: Schneider (1875) stated that trophozoites were only found in the larvae of *C. virgo*, while Geus (1969) reported their presence in both the larvae and adults of *C. virgo* (and to a lesser extent in *C. splendens*). In contrast, there are reports of an absence of infected larvae in the field (Åbro, 1971) and an inability to infect larvae with oocysts in the laboratory (Åbro, 1976). The results of this study (and those of Åbro, 1971, 1976, 1990, 1996) suggest that the adult is the primary host for eugregarine trophozoites. It therefore seems likely that the fitness consequences of eugregarine infection are restricted to the adult life-history stage of the host.

Because the fat body is responsible for producing a number of haemocoelic antibacterial peptides (Hetru *et al.*, 1998) as well as other components of the insect humoral immune system (see Brey & Hultmark, 1998), and an insect's nutritional status is known to affect immune function (Sang & Burnet, 1963), it is reasonable to assume that the size of the fat body may influence immune function and therefore parasite burdens. The notion that fat reserves are the causal agent in the negative relationship between eugregarine burdens and fat (Fig. 2) can, in this case, be rejected because the size of a male's fat body (i.e. its fat content) conferred no measurable resistance ability against eugregarine infection.

The results suggest that trophozoite infection during the teneral life-history stage results in reduced fat loads in prereproductive males, and therefore that eugregarine parasites reduce the fitness of male *C. s. xanthostoma* by reducing their ability to sustain fighting during the contests that secure and maintain ownership of a territory.

The data in Fig. 2 and the results of Plaistow and Siva-Jothy (1996) suggest that a eugregarine burden as low as two to four trophozoites reduces a preterritorial male's fat reserves to the same level as a male that has just secured a territory after an escalated contest. This is equivalent to approximately half the reserves available for territory acquisition and defence.

Eugregarines have been reported as being relatively harmless to their hosts (Steinhaus, 1947; Canning, 1956; Weiser, 1963) but studies by Åbro (1971, 1990) have shown that under heavy trophozoite burdens there are easily observed sublethal effects, including destruction of the host's mid-gut epithelium and leakage of gut contents into the haemocoel. Åbro (1971, 1990) proposed that these effects resulted in a reduction in longevity, which is an important determinant of fitness in other damselflies (e.g. Banks & Thompson, 1985).

The results presented here show that prereproductive male calopterygid hosts with relatively light burdens of eugregarine trophozoites (that are probably not manifest as measurable tissue damage) show significant reductions in fat load. Fat underpins fitness because it determines a male's ability to acquire and maintain a territory, which in turn enables males to secure matings. Low parasite burdens can therefore have major fitness consequences if they are present at critical life-history stages.

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