

BIDIRECTIONAL GENDER BIASES OF GREGARINE PARASITISM IN TWO COEXISTING DRAGONFLIES (ANISOPTERA: LIBELLULIDAE)

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Parasitism affects all taxa and influences individual and population success. Parasitism of adult dragonflies is widespread and frequently includes gregarine (Apicomplexa) life stages in the gut of the host. This research investigates variation in gregarine parasite prevalence and load in ♂ versus ♀ adults of *Erythemis simplicicollis* and *Brachymesia gravida* associated with 2 central Texas reservoirs in close proximity. Parasite prevalence was biased toward ♂ *E. simplicicollis* and toward ♀ *B. gravida*. Results suggest that gender bias in parasite prevalence is influenced by gender behavior and environment more so than by immuno-response differences between genders.

INTRODUCTION

Dragonflies process much energy in freshwater ecosystems as predatory larvae as well as adults. These predators carry a burden of gregarine intestinal parasites, but the relationships among parasite prevalence, load, and host gender are not well known. Because males and females differ in profound aspects of their biology, we must consider genders separately to understand host-parasite relationships (i.e. modes of transmission, prevalence, load, and effects on fitness) (ZUK & McKEAN, 1996). Morphological and/or physiological sexual dimorphism and sexual behavior differences often lead to differential exposure and susceptibility to parasitism (ZUK & McKEAN, 1996; SCHALK & FORBES, 1997; EDWARDS & SMITH, 2003).

Gender biases in parasite prevalence and load among vertebrates are better documented (see ZUK & McKEAN, 1996; CHRISTE et al., 2007) than for invertebrates. In general, vertebrates exhibit a male bias in susceptibility to para-

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sitism. A primary mechanism for this bias is the negative effect of androgens on the immune response in male vertebrates (GROSSMAN, 1985; ZUK & McKEAN, 1996; SCHALK & FORBES, 1997; ROBERTS et al., 2004). Gender biases are not often observed among invertebrates because they lack androgens and therefore lack a negative endocrine-immune response (ZUK & McKEAN, 1996). SHERIDAN et al.'s (2000) review of 61 studies investigating gender biases among invertebrates, for example, reports only nine cases demonstrating a gender host bias in one direction or the other.

Odonates are among the few invertebrates to exhibit gender biases in parasitism (LAJEUNESSE, 2007). HECKER et al. (2002) found a female bias in gregarine prevalence in *Enallagma boreale*, and CANALES-LAZCANO et al. (2005) reported a female bias in gregarine load for *E. praevarum*. ÅBRO (1996) found a female bias in gregarine load for *Calopteryx virgo*, and LAJEUNESSE et al. (2004) found a male bias in prevalence of the ectoparasitic mite, *Limnochares americana*, on *Leucorrhinia frigida* and *Nannothemis bella*. Several studies (ÅBRO, 1974; ANDRES & CORDERO, 1998; CORDOBA-AGUILLAR et al., 2006; LAJEUNESSE, 2007), however, report no detectable gender bias in parasitism of odonate hosts.

The purpose of this study was to determine if two common, coexisting libellulid dragonflies, *Erythemis simplicicollis* (Say) and *Brachymesia gravida* (Calvert), have gender biases as hosts for gregarine parasitism, and to discuss potential mechanisms underlying gender biases in odonates.

METHODS

Adult *E. simplicicollis* and *B. gravida* were collected with an aerial net from two reservoirs in McLennan County, Texas, USA from April – November 2007. The reservoirs, Battle Lake (BL), a small reservoir (0.32 km²) with considerable littoral vegetation, and Tradinghouse Creek Reservoir (TCR), a larger reservoir (8.14 km²) with little submerged vegetation, are 2.5 km apart. Weekly collections from BL and biweekly collections from TCR occurred between 9:00 am – 12:00 pm along the shorelines of both sites. Netted specimens were placed in glassine envelopes and taken to the lab within two hours of capture. In the lab, their abdomens were separated from the thorax and stored in 70% ethanol.

To quantify parasite prevalence and load, the preserved abdomens were placed ventral-side up on a Styrofoam tray. The abdomens were split longitudinally and pinned to expose the crops and intestines. Gregarines (trophozoites and gamonts) were visible through the intestinal epithelium and counted at 60× magnification.

Parasite prevalences were compared between males and females of each species using contingency χ^2 tests ($\alpha = 0.05$). Gregarine loads were compared between genders of each species using the non-parametric Wilcoxon Rank Sum Test because the parasite load data were not normally distributed (Shapiro-Wilk test, $p < 0.001$).

RESULTS

Combined collections at BL and TCR yielded 1,368 adults (*E. simplicicollis*, ♀ N = 327, ♂ N = 801; *B. gravida*, ♀ N = 150, ♂ N = 84). Weekly collections at BL where dragonfly populations were more abundant (personal observation) produced larger sample sizes for both species (BL: *E. simplicicollis*, ♀ N = 260, ♂ N = 760; *B. gravida*, ♀ N = 95, ♂ N = 56) compared to TCR (*E. simplicicollis*, ♀ N = 67, ♂ N = 41; *B. gravida*, ♀ N = 55, ♂ N = 28).

Analysis of combined data for the BL and TCR populations of each species revealed strong gender biases in gregarine prevalence for *B. gravida* ($\chi^2 = 12.07$, $p = 0.001$) and for *E. simplicicollis* ($\chi^2 = 14.81$, $p < 0.001$). However, the gender biases were in opposite directions for the two species. Females of *B. gravida* had a significantly greater parasite prevalence (67%) than the males (44%). In contrast, males of *E. simplicicollis* had a greater prevalence (46%) than the females (34%) (Fig. 1). When parasite prevalences for both host species were analyzed at BL and TCR separately, the female bias for *B. gravida* was consistent at the two sites (BL: $\chi^2 = 12.97$, $p < 0.001$, TCR: $\chi^2 = 5.41$, $p = 0.02$), but the male bias for *E. simplicicollis* was true only for the BL population ($\chi^2 = 5.87$, $p = 0.015$) and not the population at TCR ($\chi^2 = 1.03$, $p = 0.309$) (Tab. I).

In contrast to significant gender biases for parasite prevalence, mean gregarine loads did not differ between genders within each species (*B. gravida*: $Z = -1.722$,

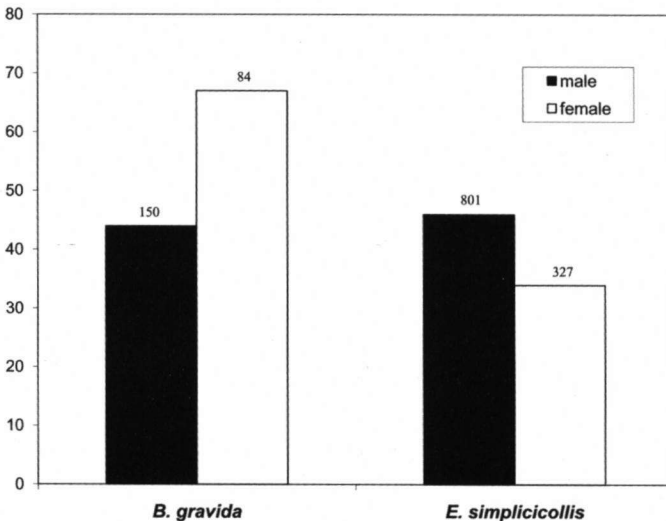


Fig. 1. Frequency of adult odonates infected with one or more gregarine parasites. Data for *B. gravida* and *E. simplicicollis* are combined samples from two central Texas reservoirs. Numbers above bars represent sample sizes.

Table I
Comparison of gregarine prevalence in the genders of *Brachymesia gravida* and
Erythemis simplicicollis

Species	Site	Gender	N	Parasite prevalence	χ^2	p
<i>B. gravida</i>	BL	Male	56	.61	12.97	<0.001
		Female	95	.86		
	TCR	Male	28	.11	5.41	0.02
		Female	55	.35		
	Combined	Male	84	.44	12.07	0.001
		Female	150	.67		
<i>E. simplicicollis</i>	BL	Male	760	.49	5.87	0.015
		Female	260	.40		
	TCR	Male	41	.05	1.03	0.309
		Female	67	.10		
	Combined	Male	801	.46	14.81	<0.001
		Female	327	.34		

$p = 0.085$; *E. simplicicollis*: $Z = -0.235$, $p = 0.814$) (Fig. 2). Mean gregarine loads for *B. gravida* and *E. simplicicollis* were 8.82 and 7.73, respectively.

DISCUSSION

At Battle Lake and Tradinghouse Creek Reservoir, the female parasitism bias of *B. gravida* was significant, yet prevalence was notably different at both sites (BL: ♀ 86%, ♂ 61%; TCR: ♀ 11%, ♂ 35%). Because these sites differ in size, they likely have different physicochemical properties. Although such properties were not measured in this study, the magnitude of the prevalence differences between the two sites suggests that habitat/environmental factors likely influence the maintenance and transmission of gregarines within dragonfly communities.

Male and female animals have different behaviors associated with feeding and reproduction, resulting in differential exposure and susceptibility to parasitism (POULIN, 1996; ZUK & McKEAN, 1996; SCHALK & FORBES, 1997; EDWARDS & SMITH, 2003; CHRISTE et al., 2007). Consequently, parasite success can vary within conspecifics due to host gender differences in encounter rates as well as the effectiveness of their immune responses (see ZUK & McKEAN, 1996; HECKER et al., 2002; CHRISTE et al., 2007; LAJEUNESSE, 2007). Such gender differences often result in differential feeding locations and rates that may increase or decrease the probability of ingesting the transmission stage and/or shift energy allocation away from immunity towards reproduction (ZUK & McKEAN, 1996).

Variation in immune responses between genders may underlie host gender

biases in parasitism. Most studies of mammals (ZUK & McKEAN, 1996; see CHRISTE et al., 2007) report a male bias in parasite prevalence and load compared to females. This bias is usually attributed to the negative influence of steroid hormones (e.g. testosterone) on the immune response. Insects, however, do not produce testosterone and consequently lack this endocrine-immune interaction. Therefore male and female insects typically do not differ in parasite prevalence or load due to immunocompetence (SHERIDAN et al., 2000). Although we found a gender bias in both odonate species studied, the biases were in opposing directions, and parasite loads did not differ between males and females for both species. If the gender biases had been in the same direction for both species or if parasite loads had differed, then it would indicate an immunocompetence difference between odonate genders and result in a universal gender bias of parasitism, i.e. a male or female bias. This was not the case, however. Our results suggest that immunocompetence does not differ between genders of these two odonate species, or is specifically ineffective against gregarine parasites for either host gender. CORDOBA-AGUILAR et al.'s (2006) study may support the latter. They found no gender bias in gregarine prevalence or load in *Hetaerina americana* and *Argia tezpi*, but based on two parameters commonly used to estimate an insect's immune system, phenyloxidase and hydrolytic lysosomal enzyme concentrations (SCHWARZENBACH & WARD, 2006; CORDOBA-AQUILAR et al., 2006), females were more immunocompetent than males.

Another explanation for host gender biases associated with parasitism is variation in host exposure to the infective parasite stage. If both genders of a spe-

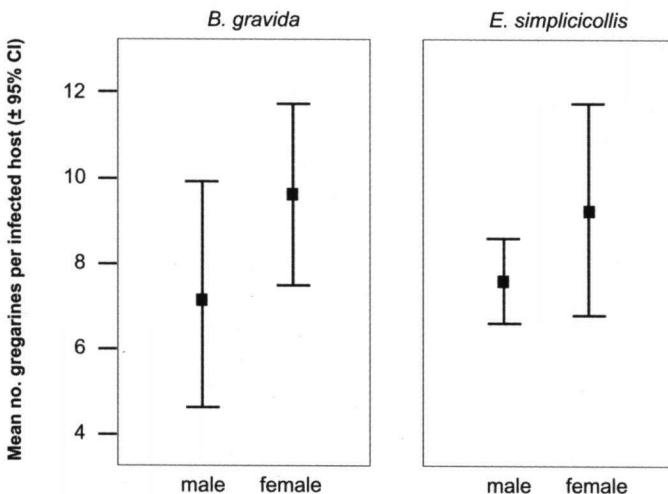


Fig. 2. Mean gregarine loads for combined samples of infected odonates from two central Texas reservoirs. Error bars are $\pm 95\%$ confidence intervals.

cies share habitats for foraging and have similar immune responses, then parasite prevalence and load patterns should be similar between genders (CANALES-LAZCANA et al., 2005). However, gender-specific behavior may expose one gender to the parasite's infective stages more so than the other gender. TINSLEY (1989) found that flukes more heavily parasitize male spadefoot toads because males spend most of their breeding season in water, whereas females visit the water only briefly to lay eggs. Such behavioral differences in odonate species could result in the bidirectional gender biases found in this study. Although general behavior has been described for *E. simplicicollis* (PAULSON, 1966; McVEY, 1981; MAY & BAIRD, 2002), little is known about *B. grandidi*. Only one study (PAULSON, 1966) briefly describes the behavior of the latter species and includes little gender-specific information. Without adequate behavioral descriptions for both species, it's difficult to clarify if the bidirectional gender biases in these two species result from differential gender exposure to the parasites.

A final question elicited by host gender biases considers mechanisms of parasite preference (an innate and active selection of a specific host to maximize fitness). In most cases of gender bias, parasites exhibit some degree of preference, as in the ectoparasitic mites parasitizing many odonate species. Larval mites attach phoretically to the final odonate instar in the water. As the odonate emerges, the larval mite transfers to the teneral and feeds on body fluids (ROLFF et al., 2001). After feeding, the mite detaches, falls back into the water, and completes its development (GRANT & SAMWAYS, 2007). Because these mites depend on aquatic habitats for life cycle completion, it's advantageous to attach to a host that either stays around water or returns to it for reproduction. Several insect studies (see EDWARDS & SMITH, 2003) report greater mite parasitism on female hosts because females usually return to water for ovipositing. McLACHLAN's (1999) report that mites on male chironomids often move to females during copulation supports this hypothesis.

Whether gregarine parasites prefer a particular host gender is not clear. Gregarine preference may not be possible because the oocysts of these intestinal parasites are ingested indiscriminately by a potential host (CLOPTON et al., 1992). The parasite would best complete its life cycle in the gender that spends the most time near water. But selection of that host gender would only be realized if the sporozoites in the ingested oocyst fail to excyst in an unpreferred host and instead pass through the digestive tract, return to the environment, and remain viable. CLOPTON et al. (1992) reported that some gregarine species are specific not only to a host species, but also to the host's life cycle stage. Excystation assays by SMITH & COOK (2008) show that gregarine excystation is species-specific in cockroaches. Although no studies quantify the concentration/availability of oocysts in the aquatic habitat, or the success rate of parasite transmission for gregarines, we assume that failure to excyst in an unpreferred gender would be a risky strategy for the parasite. Its ingestion into a second potential host of the

preferred gender may be improbable. In addition, the absence of a gregarine load difference between the genders of both species indicate that gregarine parasites lack the ability to discriminate between the genders of their hosts, or simply have no preference.

The environment, gender-specific host behavior, host immunity, and parasite preference all regulate parasite load and prevalence (ZUK & McKEAN, 1996; SCHALK & FORBES, 1997). The contrasting gender biases found in this study do not fully distinguish among these factors. They do, however, bring focus to some paths for research investigating factors associated with the occurrence and maintenance of gregarine parasites in odonate communities.

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