

# On understanding variation in immune expression of the damselflies *Lestes* spp.

C.P. Yourth, M.R. Forbes, and B.P. Smith

**Abstract:** Immune ability and immune expression have been viewed as life-history traits that are influenced by such factors as the likelihood of being parasitized, intensity and costs of parasitism, and trade-offs associated with immune expression. In this paper we show that different patterns of infestation by a generalist ectoparasite, *Arrenurus planus* Marshall (Arrenuridae: Hydrachnida), do not fully explain the variation in immune expression across four species of sympatric damselflies (Lestidae: Zygoptera). Within species, no gender biases in immune expression were evident. Whereas both males and females of one oft-exploited species did not mount immune responses against attending larval mites, males and females of three other species showed similar immune responses, with variable expression. The immune response was melanotic encapsulation of mite feeding tubes, and was associated with dead mites. Of the three species showing immune expression, the species with the highest prevalence and intensity of infestation had a significantly higher proportion of individuals responding immunologically to mites. In conclusion, current infestation levels only partially predict immune investment; consideration of the timing of emergence of different species suggests that season may be an important predictor of immune investment.

**Résumé :** La capacité immunitaire et l'expression de l'immunité sont parfois considérées comme des caractéristiques de la biologie d'une espèce, caractéristiques influencées par divers facteurs, tels que la probabilité, la gravité et les coûts du parasitisme, de même que les compromis associés à l'expression de l'immunité. Nous démontrons ici que les différents patterns d'infestation d'un ectoparasite généraliste, *Arrenurus planus* Marshall (Arrenuridae : Hydrachnida), ne suffisent pas à expliquer totalement la variation dans l'expression de l'immunité chez quatre espèces sympatriques de demoiselles (Lestidae : Zygoptera). Au sein des espèces, nous n'avons pas constaté de tendance particulière des mâles ou des femelles dans l'expression de l'immunité. Bien que les mâles et les femelles d'une espèce souvent exploitée ne développent pas de réaction immunitaire contre les larves d'acariens présentes, les mâles et les femelles de trois autres espèces réagissent de façon semblable, mais avec des variations dans l'expression de leur immunité. La réaction immunitaire résulte en l'encapsulation mélanique des tubes alimentaires des acariens, phénomène associé aux acariens morts. Des trois espèces dont l'immunité s'exprime, l'espèce qui a le plus haut taux de prévalence et subit les infections les plus graves a une proportion significativement plus élevée d'individus qui réagissent à la présence des acariens. Il semble donc que les taux d'infestation enregistrés à un moment donné ne permettent pas de prédire entièrement l'investissement immunitaire; l'examen du synchronisme de l'émergence de différentes espèces indique que la saison peut être un facteur important de prédiction de l'investissement immunitaire.

[Traduit par la Rédaction]

## Introduction

One main goal of the emerging subdiscipline of ecological immunology is to understand which (or when) hosts should commit resources, time, and energy to defense against parasites and pathogens (Sheldon and Verhulst 1996). Typically, studies on variation in resistance or in susceptibility are done at the within-species level. We suggest that studies of generalist parasites attacking suites of closely related species can present another approach to testing the theory and targeting questions of interest for further within-species stud-

ies. We illustrate these points through a detailed study of the extent to which males and females of four related and syntopic species of damselflies (Lestidae: Zygoptera) are differentially parasitized by the ectoparasitic water mite *Arrenurus planus* Marshall (Arrenuridae: Hydrachnida). We then test whether differential parasitism is predictive of differential immune responses.

This central prediction is easily tested using our study system. Larval mites colonize larval hosts before actual eclosion to adults, and host responses to mites occur before host reproduction. In damselflies, the host response is melanotic encapsulation of mite feeding tubes (Åbro 1979, 1982). For damselflies, any variable responses among species should not be confounded by the following: differences in timing of parasitism or variation in reproductive effort. Others have studied whether differences in age-specific reproductive effort of hosts carry a cost of increased parasitism: some observational and experimental studies provide support, whereas others do not (reviewed in Møller 1997).

Another factor deemed important to immune responses is the cost of parasitism. The physiological costs of parasitism

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**C.P. Yourth and M.R. Forbes.**<sup>1</sup> Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada.

**B.P. Smith.** Biology Department, Ithaca College, 953 Danby Road, Ithaca, NY 14850-7278, U.S.A.

<sup>1</sup>Corresponding author (e-mail: mforbes@ccs.carleton.ca).

by individual larval mites are probably similar for the damselfly species considered herein, for two reasons. First, only one species of parasite is being considered. In fact, *A. planus* is the *only* mite species exploiting these damselflies at our study site. Second, there is considerable size overlap between these damselfly species (Walker 1953). Notwithstanding, the costs of parasitism *may* differ among species depending on differences in intensity of infestations. Thus, we consider not only the prevalence of parasitism, but also the intensity of infestation once the damselflies were parasitized.

We note that the absolute costs of resistance should be similar across species if the same immune mechanism is deployed (i.e., melanotic encapsulation of feeding tubes). The cost of resistance is often viewed as a fundamental aspect to be considered in ecological immunology. The cost of resistance for insect hosts has been shown or strongly inferred using different approaches (e.g., König and Schmid-Hempel 1995; Kraaijeveld and Godfray 1997; Siva-Jothy et al. 1998; Fellowes et al. 1999). Although the same resistance mechanism is deployed in damselflies, its deployment may have varying costs across species because of differing trade-offs mediated by unknown ecological factors. We note that comparisons of species differing in their ecology may provide clues about trade-offs with immunity.

We scored differences in parasitism by mites in two ways: calculating the proportion of hosts infested and the numbers of mites on infested hosts (termed prevalence and intensity, respectively; *sensu stricto* Bush et al. 1997). In relation to our main objective we also considered emergence dates of hosts. Temporal co-occurrence of hosts and larval mites has been implicated in host-species use in the case of other mite–insect systems (e.g., Smith and McIver 1984a; Forbes and Baker 1991). We included seasonal data to see if we could explain variation in prevalence of parasitism. Irrespective of the initial cause(s) of differences in parasitism, our main objective was to investigate whether immune expression (the proportion of hosts showing some degree of response) could be predicted by current measures of parasitism. We do not test the corollary: whether better defended hosts have fewer mites because mites induce defense. Better defended hosts, therefore, should have higher numbers of dead mites, but not necessarily fewer mites.

### Relevant natural history of the host–parasite association

Herein we examine the suitability of four lestids (*Lestes congener* Hagen, *Lestes dryas* Kirby, *Lestes forcipatus* Rambur, and *Lestes unguiculatus* Hagen) as hosts for the water mite *A. planus*. *Arrenurus planus* is a generalist parasite, exploiting a small number of dragonfly species in the genus *Sympetrum* and a similarly small number of damselfly species in the genus *Lestes*. While only a handful of species in two disparate taxa are parasitized, the link is that these hosts are the only odonates in the region that inhabit temporary autumnal and vernal woodland pools (Wiggins et al. 1980).

We first compared prevalences of attached mites and intensities of infestation, and then compared successful engorgement by attached mites, between males and females and among species. These four species emerged from the same pond and were parasitized by only one mite species,

*A. planus*. In fact, *A. planus* is the only parasite of odonates in temporary waters, and is an obligate temporary-pool specialist.

Colonization of odonate hosts by *Arrenurus* spp. occurs in a series of steps during which the hosts may mount defenses. Larval mites first colonize odonate larvae; however, larvae groom and can reduce attachment rates (Forbes and Baker 1990; Leung et al. 1999). Larval mites are phoretic on odonate larvae; once the larval hosts emerge and start to eclose, the larval mites abandon the cast exoskeleton and crawl onto the newly formed imago. The mites cling to the host with their palpal claws, and soon pierce the host's cuticle using their chelicerae (Smith 1988). Anchored to the host by the chelicerae, the mite secretes an acellular mucopolysaccharide that forms a stylostome (a blind-ended feeding tube; Smith 1988).

As mentioned, some dragonfly species attempt to neutralize one or more stylostomes through melanotic encapsulation, which occurs when the stylostome first penetrates the cuticle (Åbro 1979, 1982). In other species such as *Sympetrum internum* Montgomery, the host appears to aggregate haemocytes at the site of stylostome formation (Forbes et al. 1999), like other insects parasitized by mites (cf. Davids 1973). If resistance is not mounted and the mite engorges successfully, it drops off the host when the host returns to the water for reproduction, which is similar to other mite–damselfly associations (Rolff and Martens 1997). The mite then continues through its nymphal and adult stages as a free-living aquatic predator on microcrustaceans. On some hosts, scars are clearly visible following mite detachment (Forbes 1991). In this study, damselflies that had just acquired their mites (newly emerged) are included; damselflies that had matured and had mites but no mite scars are also included (but see below for an exception). Thus, we could test whether damselflies emerging with mites exhibited melanotic resistance, whether such resistance killed mites, and whether infestation or the magnitude of infestation was predictive of immune investment.

## Methods

### Censuses to examine patterns of mite infestation and emergence dates

We caught teneral or newly emerged damselflies and reproductively mature damselflies to elucidate patterns of infestation and engorgement success. Collections were done at Yezerinac's Pond, located ca. 6 km southwest of the Queen's University Biological Station (QUBS) facilities on the Hilda and John Pangman Conservation Reserve. QUBS is located ca. 2 km west of Chaffey's Lock, Ontario, Canada (44°34'N, 79°15'W). We made 51 trips to Yezerinac's Pond between 28 May and 5 August 1998. On each trip we netted and then visually inspected damselflies for species identification (following Walker 1953) and to count any mites, using a 20× loupe. We recorded gender and approximate age of each individual (cf. Walker 1953). In all species, tenerals had soft and shiny wings, a soft abdomen, and little dark pigmentation in the exoskeleton. Young to old adults all had rigid and dull wings, young adults had melanin deposits in their appendages only, older adults had body pigmentation, and the oldest adult males were pruinose.

In total, 754 damselflies were used in analyses. We marked damselflies on the wings after processing them as described below, to preclude their resampling (following Forbes 1991). Seventeen damselflies were old adults (5 *L. unguiculatus*, 2 *L. congener*,

10 *L. forcipatus*) and had only scars and no mites. These were excluded from analyses because we could not score mite numbers reliably. Another 23 adults (ca. 3%) had a few scars and mites, which were identified as *A. planus* (1 *L. unguiculatus*, 3 *L. congener*, 19 *L. forcipatus*). Thus, a few mites appeared to have detached. We included these adults in our analyses because they still had many *A. planus*, which appear to detach more or less simultaneously (i.e., only 3% of adults were found with both scars and mites).

For female *L. congener*, 14 teneral and 11 older adults were caught, and for male *L. congener*, 9 teneral and 18 older adults were netted. More individuals of the remaining species were netted. For female and male *L. dryas*, 24 and 23 teneral and 33 and 75 older adults, respectively, were caught. For female and male *L. forcipatus*, 4 and 1 teneral and 37 and 49 older adults, respectively, were caught. Finally, for female and male *L. unguiculatus*, 53 and 54 teneral and 150 and 182 older adults, respectively, were caught. These differences in sample size may reflect differences in availability at times of sampling rather than differences in actual density.

For analyzing emergence dates, *only* teneral were included (based on aging criteria above); these emerged within 1–2 days of collection. For all other analyses, all age categories were considered. To score various aspects of parasitism and engorgement success of parasites, we recorded the numbers and locations of all *A. planus* found on teneral damselflies and their engorgement success on older damselflies. Mites were almost invariably found on the venter of the thorax on a tubercle, or near or on the coxal plates of the legs. Dead mites had a flattened body that was silver in colour, indicating that it had not engorged and that death had occurred shortly after attachment. Mites starting to engorge had a red to orange body and their legs were still visible. Mites were scored as fully engorged if their legs were entirely obscured by their swollen body and partially engorged if the legs were only partially obscured by the body (cf. Forbes et al. 1999).

### Viewing mite stylostomes or feeding tubes

We brought 39 damselflies with one or more dead mites back to the laboratory. We prepared samples for viewing stylostomes of mites (7 and 6 female and male *L. congener*, respectively; 14 and 11 female and male *L. forcipatus*, respectively; and 1 female *L. unguiculatus*). No *L. dryas* were included in this part of the study because none were found with dead mites. We stored these damselflies in glassine envelopes and refrigerated them until they were processed (within 48 h). Preparation involved removing the head, legs, wings, and abdomen of the damselfly, leaving only the thorax. We placed the thoraces in a 4.5 × 1.5 cm glass vial and covered them with Andre's solution (1:1:1 chloral hydrate : acetic acid : water by mass). We then stored the vials for a minimum of 48 h at room temperature (ca. 20°C) before conducting the dissections.

To dissect the damselflies we removed the top half of the thorax (including the wing muscles) using No. 5 forceps and a 2.5-cm 22-gauge syringe needle. We then placed the ventral half of the thorax on a microscope slide (with the exocuticular surface touching the slide). After applying glycerol to the sample and covering it with a cover slip, we examined each slide at 100× (phase contrast) magnification and visually scanned for mites and stylostomes. We captured digital images of a subset of stylostomes that appeared to be whole, using a video camera mounted on a microscope and Snappy® software (Play Inc. 1996. Snappy Video Snapshot, Rancho Cordova, U.S.A.).

To examine variation in successful engorgement, we counted dead mites found on mature males and females of the different host species. To see if the presence of dead mites was the result of immune responses by the hosts, we examined whether dead mites were associated with partially or wholly encapsulated stylostomes,

using the subsamples of the three host species. Pictures of melanized stylostomes are available from the authors on request.

### Analyses

We used  $\chi^2$  tests to determine whether prevalence of infestation depended on gender within species or on species (when males and females within species were combined). We made further among-species comparisons by calculating 95% confidence limits around prevalence estimates (after Snedecor and Cochran 1980).

We tested the distribution of numbers (and log-transformed numbers) of mites for deviation from normality using Kolmogorov–Smirnov tests. We used Kruskal–Wallis tests for subsequent comparisons of median intensities of infestation for males and females within species and for males and females combined across species. We made further comparisons among species using Dunn's multiple-range test for unequal sample sizes, correcting for ties (Zar 1996).

We compared emergence dates using a two-way ANOVA with gender and species as the main factors, and made post-hoc comparisons of mean emergence dates for the different species using Tukey's test.

Finally, we tested for differences in host immune responsiveness between males and females of each species and for males and females combined across species by means of  $\chi^2$  tests. Further among-species comparisons were made by comparing 95% confidence limits around estimates of the proportion responding to mites.

## Results

### Do males and females or species differ in mite infestation?

Generally, there were no gender biases in prevalence for each species considered separately, although female *L. dryas* tended to become more infested than male *L. dryas* ( $\chi^2 = 3.52$ ,  $df = 1$ ,  $P = 0.06$ ; Table 1). In comparison, species clearly differed in the probability of becoming infested with mites when males and females were combined ( $\chi^2 = 237.4$ ,  $df = 3$ ,  $P < 0.001$ ). All species differed from one another in this respect, as the 95% confidence limits did not overlap for any pairwise comparison between species (Table 1). *Lestes unguiculatus* had the lowest prevalence overall (9.8%, sexes combined), followed by *L. dryas* (23.2%), then *L. congener* (46.2%), and finally *L. forcipatus* (83.5%). Thus, we predicted that the degree of immune expression might follow the same pattern if prevalence of infestation alone could select for more or less maintenance or expression of immunity.

Numbers (and log-transformed numbers) of mites on hosts were non-normally distributed, precluding parametric tests for differences in mean numbers of mites between categories of hosts. Median intensities of mite infestation did not differ between females and males of *L. congener*, *L. forcipatus*, and *L. unguiculatus*. In comparison, female *L. dryas* carried significantly more mites than male *L. dryas* ( $\chi^2 = 5.72$ ,  $P < 0.025$ ; Table 2). We excluded *L. dryas* from subsequent statistical comparisons of intensity between species, since the variation in infestation within this species was considerable. Male *L. dryas* had a median intensity of one mite, which was similar to male and female *L. unguiculatus*; female *L. dryas* had a median intensity higher than that of males and females of both *L. unguiculatus* and *L. congener* (Table 2).

As in the prevalence data, the remaining three species showed considerable variation in median intensity of mite infestation ( $\chi^2 = 35.3$ ,  $P < 0.001$ ). We found that median intensities of infestation differed significantly *only* between

**Table 1.** Numbers of damselflies (by species and gender) that were uninfested ( $N_u$ ) versus infested by one or more mites ( $N_i$ ), prevalence of infestation, and the confidence interval (CI) calculated following Snedecor and Cochran (1980) and Bush et al. (1997).

	$N_u$	$N_i$	Prevalence (%)	CI	$\chi^2$	$P$
<i>Lestes congener</i>						
Females	12	13	52.0	19.6	0.67	0.42
Males	16	11	40.7	18.5		
Combined	28	24	46.2	13.5		
<i>Lestes dryas</i>						
Females	39	18	31.6	12.1	3.52	0.06
Males	80	18	18.4	7.7		
Combined	119	36	23.2	6.6		
<i>Lestes forcipatus</i>						
Females	7	34	82.9	11.5	0.019	0.89
Males	8	42	84.0	10.2		
Combined	15	76	83.5	7.6		
<i>Lestes unguiculatus</i>						
Females	182	21	10.3	4.2	0.13	0.72
Males	214	22	9.3	3.7		
Combined	396	43	9.8	2.8		

**Note:** Chi-squared-test statistics and associated  $P$  values are presented for comparisons between males and females separately for each species. Combined values for males and females of each species are also presented. See the text for further statistical comparisons among species.

**Table 2.** Median numbers of mites (*Arrenurus planus*;  $M$ ), and interquartile ranges (IQR) and full ranges (FR) for both sexes of each species.

	$N$	$M$	IQR, FR	$\chi^2$	$P$
<i>Lestes congener</i>					
Females	13	2	1–3, 1–6	0.004	0.95
Males	11	2	1–5, 1–13		
<i>Lestes dryas</i>					
Females	18	3	1–4, 1–10	5.72	0.017
Males	18	1	1–2, 1–9		
<i>Lestes forcipatus</i>					
Females	34	4	2–7, 1–27	0.50	0.48
Males	42	5	2–9, 1–17		
<i>Lestes unguiculatus</i>					
Females	21	1	1–2, 1–3	0.016	0.90
Males	22	1	1–2, 1–7		

**Note:**  $N$  is the sample size used for calculating medians and ranges;  $\chi^2$  test statistics and associated  $P$  values refer to the Kruskal–Wallis test comparing median intensities of infestation between females and males separately for each species. See the text for statistical comparisons among species. N.B. Only hosts with one or more mites were included in the calculations.

*L. forcipatus* and *L. unguiculatus* ( $P < 0.05$ ). Thus, while the prevalence data clearly predicted variation in immune expression, the intensity data suggested that *L. forcipatus* would differ from *L. unguiculatus*. The prediction was less clear with respect to the other species, *L. congener* and *L. dryas*. Taken together, species clearly differed in prevalence of infestation, but only some species were significantly different with respect to intensity of infestation.

#### Do emergence dates vary across species and does this account for the variation in mite prevalence?

We found significant variation in emergence dates (based

on dates of capture of teneral) across species (species:  $F_{[3,173]} = 239.5$ ,  $P < 0.001$ , overall  $r^2 = 0.81$ ). Gender and the interaction between gender and species did not account for significant variation in emergence dates (gender:  $F_{[1,173]} = 0.26$ ,  $P = 0.68$ ; interaction:  $F_{[3,173]} = 0.51$ ,  $P = 0.68$ ). For *L. dryas*, the emergence date was  $153.7 \pm 0.8$  (mean  $\pm$  SE) days (day 1 = 1 January 1998), whereas for *L. forcipatus* and *L. unguiculatus*, the emergence date was  $164.0 \pm 2.7$  and  $170.3 \pm 0.5$  days, respectively. *Lestes congener* emerged the latest of all species: its emergence date was  $190.4 \pm 1.1$  days. *Lestes forcipatus* and *L. unguiculatus* emerged midseason and showed no significant difference in mean emergence dates. In comparison, *L. dryas* emerged significantly earlier than these two species and *L. congener* emerged significantly later than all three other species.

Across species, emergence date did not appear to account for the significant variation in prevalence of parasitism by mites, in contrast to the results of other studies. In fact, the two species that overlapped in emergence dates (*L. forcipatus* and *L. unguiculatus*) showed the greatest difference in prevalence.

#### Are dead mites associated with melanized stylostomes?

As indicated, no male or female *L. dryas* were found with dead mites and so were excluded from stylostome preparations made to examine whether dead mites were associated with encapsulated stylostomes. We made 39 preparations of the individuals that were brought back to the laboratory with one or more dead mites. We were able to view stylostomes associated with 86 dead mites, all of which were associated with partially (>50%) or wholly melanized stylostomes. These results indicate that males and females of both *L. congener* and *L. forcipatus* are able to mount effective immune responses to mites, as are female *L. unguiculatus* (no male *L. unguiculatus* were prepared for viewing stylostomes). As

**Table 3.** Immune expression in males and females of each species.

	$N_m$	$N_r$	$P_r$	CI
<i>Lestes congener</i>				
Females	13	5	38.5	26.4
Males	11	7	63.6	28.4
Combined	24	12	50.0	20.0
<i>Lestes dryas</i>				
Females	18	0	0	0
Males	18	0	0	0
Combined	36	0	0	0
<i>Lestes forcipatus</i>				
Females	34	25	73.5	14.8
Males	42	30	71.4	13.7
Combined	76	55	72.4	10.1
<i>Lestes unguiculatus</i>				
Females	21	7	33.3	20.2
Males	22	7	31.8	19.5
Combined	43	14	32.6	14.0

**Note:**  $N_m$  is the total number of damselflies with one or more mites;  $N_r$  is the number of damselflies with one or more dead mites, resulting from encapsulation of mite feeding tubes;  $P_r$  is the proportion of hosts responding immunologically; CI is the confidence interval around that proportion, following Snedecor and Cochran (1980). Combined values for males and females of each species are also presented.

indicated, male and female *L. dryas* showed no evidence of melanotic immunity. Many damselflies of the other three species had both live and dead mites. Some mites had perfectly formed stylostomes with no evidence of melanization on the stylostome itself. Such mites were alive before preparations were made.

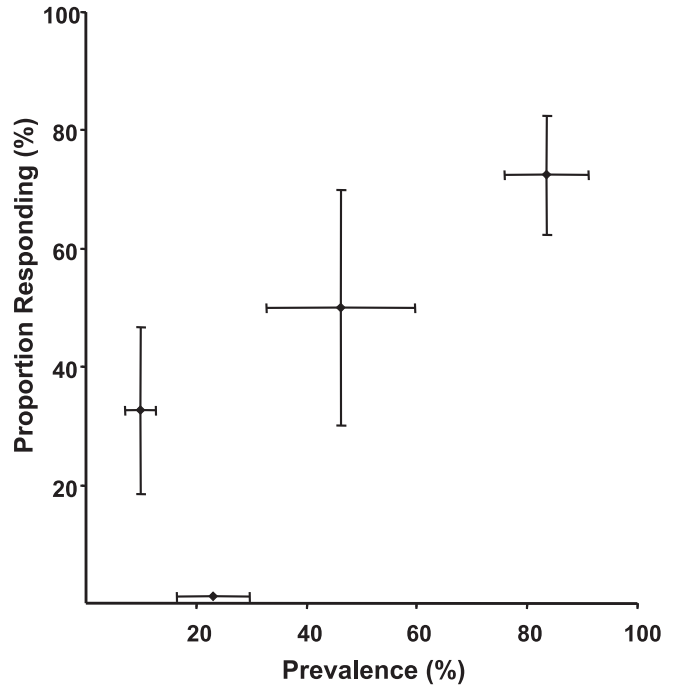
**Does immune responsiveness vary across species?**

There were also no differences between males and females in the proportion of hosts with one or more dead mites ( $\chi^2$  values ranged from 0 to 0.5 and  $P$  values from 0.48 to 1.0; Table 3). When males and females were combined, we found significant variation in the proportion of hosts with one or more dead mites ( $\chi^2 = 55.3$ ,  $df = 3$ ,  $P < 0.001$ ). When we excluded *L. dryas* from tests, we still found significant variation across species in the proportion of hosts responding ( $\chi^2 = 18.2$ ,  $df = 2$ ,  $P < 0.001$ ). *Lestes forcipatus* responded to mites more than *L. unguiculatus*, although *L. forcipatus* and *L. congener* did not differ in this respect (compare 95% confidence limits for combined values from Table 3; see also Fig. 1).

**Discussion**

Several factors, such as costs of parasites and costs of resistance, should dictate whether or not a species evolves the ability to respond immunologically to a given parasite. In this study, we assessed whether current patterns of infestation by a generalist ectoparasite might help to explain immune expression across four species of lestad damselflies. We chose this study system because only one mite species exploited all host species. In addition, resistance to *Arrenurus* spp., if deployed, is similar among damselfly

**Fig. 1.** Proportions (with confidence intervals) of individuals responding in relation to prevalence (with confidence intervals) of mite infestation for the damselflies *Lestes unguiculatus*, *Lestes dryas*, *Lestes congener*, and *Lestes forcipatus* (from left to right). Confidence intervals were calculated following Snedecor and Cochran (1980). Note that the proportion of individuals responding is zero for *L. dryas*.



hosts (but for a different mechanism of resistance in a dragonfly see Forbes et al. 1999).

To our knowledge, no one has examined, theoretically or empirically, the relative importance of prevalence and intensity of infestation in the evolution of immunity or degree of immune expression. These two metrics of parasitism are generally related across species (Bush et al. 1997), and specifically in water mite – insect associations (Smith 1988). However, they need not relate in such a way that the relative positioning of host species is the same for both measures (this study).

Based solely on the probability of infestation, we predicted that immune expression would be greatest in *L. forcipatus*, followed by *L. congener*, then *L. dryas*, and least likely in *L. unguiculatus*. Based on these results on prevalence, combined with those on intensity of infestation, we predicted that *L. forcipatus* would show the greatest immune expression. Immune expression in *L. forcipatus* should exceed that in *L. unguiculatus* (because its prevalence and intensity of infestation are higher than in *L. unguiculatus*) and perhaps *L. congener* (because *L. forcipatus* had a higher prevalence, although not a higher intensity of infestation).

Our general results on immune expression were quite interesting. First, all 86 dead mites examined had partially or wholly encapsulated stylostomes, i.e., none had fully formed stylostomes and appeared to have died from other causes. Thus, all dead mites occurred as a result of host immune responses. Since encapsulation of the stylostome, and thus the death of the mite, occur at or soon after the time of attachment, our study is not confounded by the age of the host

when it was sampled. Second, at least some mites successfully engorged on all four species. In fact, all mites found on *L. dryas* had encountered no resistance. That *L. dryas* showed no immune expression does not support our general prediction because hosts that were used less often showed resistance. The general prediction was not supported for another reason: the responsiveness of *L. congener* overlapped the high responsiveness of *L. forcipatus* as well as the low responsiveness of *L. unguiculatus*. It is important to note, however, that *L. congener* differed from the other two species in prevalence of parasitism only, and not in intensity.

In summary, our intent was to test first for differences in prevalence or intensity of infestation, and then whether these were predictive of differences in immune investment among the four syntopic damselfly species. All four species aligned themselves in terms of prevalence of infection, but not intensity. If the same ranking of prevalences was observed for immune investment, it would represent only 1 out of 24 possible rankings of species ( $0.01 < P < 0.05$ ). Thus, our results do not suggest that prevalence, by itself, is strongly predictive of immune investment. Yet we found clear differences in prevalence and intensity of infestation *only* for the comparison between *L. forcipatus* and *L. unguiculatus*, and this single comparison supported the general prediction. Furthermore, these species overlapped in timing of emergence, which we now hypothesize to be an important determinant of immune investment.

We support this contention and our general approach for several reasons. First, we know of no other example of a direct comparison of degree of parasitism and immune investment across species (although a few other studies have compared proxies of immunity; e.g., Møller and Erritzøe 1996, 1998). Rather, the effects and consequences of parasitism are often studied with single host species. Tests with generalist parasites that affect several host species from the same locale are desperately needed. If we have many such tests, we may get a clearer picture of factors that influence parasitism and immune investment. The species we studied are closely related and similar, but also show some differences in their ecology. It is important to ask whether these differences might be predictive of parasitism and (or) immune investment.

We found, for example, that emergence date was not a strong predictor of patterns of infestation, contrary to other work (see references in the Introduction). In other insect-mite systems, early-emerging host species may actually avoid parasites (Smith and McIver 1984a). When temporal barriers are removed in the laboratory, early-emerging mosquito species are more susceptible to mite colonization than later emerging species, which normally overlap temporally with the mite species (Smith and McIver 1984b). In this study, the early-emerging species was not resistant to mites. We are currently examining whether expression of resistance is season-dependent in species for which we have sufficient data. For *L. forcipatus*, work from our laboratory has shown that later emerging individuals are significantly more likely to respond immunologically (C.P. Yourth, M.R. Forbes, and B.P. Smith, unpublished data). This study was a direct consequence of observing species differences in immune investment in relation to date.

We still need to explain such seasonal variation, and here we present a testable hypothesis: while the actual costs of

melanin production and mobilization may be the same among species, species might otherwise vary in trade-offs associated with this immune response. For example, melanin is used in thermoregulation by damselflies (Corbet 1999). Early-emerging species may be subjected to more periods of cool wet weather (Corbet 1999) and for this reason may be less willing to surrender melanin resources to immune responses. This interesting hypothesis of a trade-off with thermoregulation and its effect on maturation rate has not been explored for any insect host that uses melanin to combat parasites. Alternatively, immune expression may simply be temperature-dependent, a hypothesis that we intend to investigate further.

One final problem is worth considering. As mentioned, *A. planus* also exploits dragonflies of the genus *Sympetrum* (Forbes et al. 1999); this indicates that host phylogeny is not a strong impediment to host exploitation. We expect that host representation may vary across ephemeral ponds that are habitats for *A. planus*. Data are needed on spatial variation in host-parasite overlap. Such studies will help us understand whether variation in use of particular host species is predictive of a host's ability to respond to *A. planus* or the strength of any responses. Geographical patterns of infestation and immunity could prove as illuminating as seasonal comparisons.

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