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Resistance to *Arrenurus* spp. Parasitism in Odonates: Patterns Across Species and Comparisons Between a Resistant and Susceptible Host

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Abstract

Some adult odonates resist parasitism by larval water mites (*Arrenurus* spp.) with melanotic encapsulation, in which the mite's stylestome is clogged and the mite starves. In summer 2014, we counted the engorged and resisted mites on 2,729 adult odonates sampled by aerial net at 11 water bodies in Greenville Co. and Pickens Co., SC, and tested the hypothesis that the frequency and intensity of resistance correlates with parasite prevalence (the percentage of parasitized hosts). Resistance prevalence (the percentage of parasitized hosts that resisted at least one mite) varied significantly among host species, exceeding 60% for *Argia fumipennis* (Burmeister) and *Celithemis fasciata* Kirby but less than 20% for other species. However, neither resistance prevalence nor mean resistance intensity (mean percentage of resisted mites on resisting hosts) correlated with parasite prevalence. We described potential effects of parasitism on host development of *A. fumipennis* and *Pachydiplax longipennis* (Burmeister) by comparing the percent asymmetry of forewing lengths between parasitized and unparasitized individuals. There was no significant difference in asymmetry for either males or females of *A. fumipennis*, or males of *Pa. longipennis* (females were not sampled). We also evaluated differences in melanotic encapsulation between *A. fumipennis*, which readily encapsulates mites in nature, and *Pa. longipennis*. We inserted a 2.0-mm piece of sterile monofilament line into the thorax of captured individuals for 24 h and compared mean gray value scores of inserted and emergent ends using Image-J software. There was no difference in melanotic encapsulation between species.

Key words: *Arrenurus*, *Argia fumipennis*, *Pachydiplax longipennis*, parasitism, parasite resistance

Parasites can affect the physiology, morphology, behavior, and fitness of their hosts (Goater et al. 2014). Consequently, they can affect competitive and predatory outcomes between their hosts and other species, affecting community dynamics and patterns in biodiversity (Hatcher and Dunn 2011). The first step in understanding the potential effects that parasites might have on community structure is to survey the distribution of parasitism across co-occurring species and identify patterns of parasitism, host susceptibility, and host resistance.

Dragonflies and damselflies (Order: Odonata) are excellent model systems for understanding the effects of parasitism on hosts and host communities. Like many aquatic insects, odonates are parasitized by larval water mites from the families Arrenuridae, Hydraphantidae, and Limnocaridae (Smith and Oliver 1986). Hydraphantids and limnocarids have terrestrial larvae that either leap from the surface of the water to infect mature odonates in flight (Corbet 1999, p. 326) or colonize odonates perching along the shoreline (Smith 1988). Arrenurids have aquatic larvae; most species colonize odonate naiads and begin to feed on the emerging adult at ecdysis, but some species colonize mature odonates returning to water to mate or oviposit (Corbet 1999, p. 324). The parasite pierces the host with palpal claws and feeds through a secreted feeding tube ("stylostome") on dissolved host tissue and hemolymph (Smith 1988). After approximately 10 d of feeding, mites drop off the host into the water to complete their life cycle—preying on ostracods and other microcrustaceans as adults (Forbes and Robb 2008). As much as 50% of a parasite's growth can occur during the parasitic larval stage (Zawal and Buczyński 2013).

Individual odonates can be parasitized by over >400 mites at one time (Worthen and Turner 2015), from as many as nine mite species (Zawal and Buczyński 2013). Parasite prevalence can reach 100% for some species in particular habitats (Zawal and Buczyński 2013). Parasitism by mites causes tissue damage (Åbro 1982), reduced host mass (Smith 1988), and reduced survivorship (Forbes and Baker 1991, Leung and Forbes 1997), and changes in flight behavior (Reinhardt 1996, Nagel et al. 2011). Mites can reduce

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reproductive success (Forbes 1991) and have direct effects on fitness in some species (see review by Forbes and Robb 2008), as parasitized females may lay fewer eggs (Rolff 1999, Rolff et al. 2001) and parasitized males may have smaller testis and produce less effective sperm (Rolff et al. 2001). Mite parasitism can also affect developmental stability in some host species. For example, body symmetry in *Coenagrion puella* (as indicated by similarity in forewing lengths) is inversely correlated with the number of mites present (Bonn et al. 1996).

Some odonate species respond to mite parasitism through melanotic encapsulation: a generalized immunological response common to many invertebrates (Götz and Boman 1985) in which the hemocytes encapsulate and clog the stylestome. The mites, stuck and unable to feed, become "deflated" and die (Nagel et al. 2011), providing a visible index of host resistance. This response can be induced in some species by inserting a thin piece of monofilament line into the thorax (Nagel et al. 2011).

In this investigation, we examined the relationships between mite parasitism and host resistance three ways. First, we extended the analysis of a previous survey of parasite prevalence (Worthen and Turner 2015) and tested the hypothesis that more frequently parasitized species are more likely to mount an immunological response. Second, we measured the forewings of captured individuals and tested the hypothesis that an immunologically resistant species, *Argia fumipennis* (Burmeister), would be affected by parasitism (as indicated by forewing symmetry) less than *Pachydiplax longipennis* (Burmeister), which resists mites less frequently. Finally, we conducted a laboratory experiment to elicit resistance responses to artificial inserts (following Nagel et al. 2011). We tested the hypothesis that *A. fumipennis*, the more resistant species in nature, would more strongly encapsulate an artificial insert than *Pa. longipennis*.

Materials and Methods

Survey of *Arrenurus* Parasitism and Host Resistance

Odonates were collected by aerial net at 11 waterbodies in Greenville Co., SC, and Pickens Co., SC, at approximately weekly intervals, from May to August 2014. Sites ranged in size and ecology from Lake Oolenoy at Table Rock State Park—which is large (27.1 ha), deep, and surrounded by intact woodland—to a small (0.1 ha), shallow, semipermanent pond at Bunched Arrowhead Heritage Trust Preserve that is surrounded by successional fields (for a complete description of the sites and their location coordinates, see Worthen and Turner 2015). We collected 15–30 individuals during each sampling event, identified the species and sex of each individual, and used a 20× loupe to count the engorged and resisted *Arrenurus* mites on each. A wing was labeled with a site number before release to prevent double counting if recaptured. For *A. fumipennis* and *Pa. longipennis* individuals, the length of each forewing was measured three times using a digital caliper (0.01 mm resolution, EZ Cal caliper, iGaging Precision Tools & Gauges, San Clemente, CA).

Patterns in mite parasitism and resistance were analyzed several ways. Differences among species in parasite prevalence (percentage of individuals with at least one mite) were analyzed with a chi-squared test, using only species with mites or with >30 individuals captured (for a complete list of species captured, and a more complete analysis of patterns in parasite prevalence, see Worthen and Turner 2015). Differences in parasite intensity (mean number of mites per parasitized individual) among species were analyzed with a Kruskal–Wallis test, including only the species with at least 10 parasitized individuals. Resistance was also measured in terms of prevalence (percentage of parasitized individuals with at least one resisted mite) and intensity (mean percentage of mites that were resisted on individual hosts that resisted at least one mite). Differences in resistance prevalence were analyzed using a chi-squared test, including only species with at least 10 parasitized individuals. Resistance intensity was analyzed using a Kruskal–Wallis test, including only the species with at least three individuals that resisted at least one mite. We also compared the prevalence and intensities of parasitism and resistance between sexes with chi-square and Mann–Whitney *U* tests, where sample sizes permitted. Nonparametric tests were used to compare intensities because the numbers of mites and resisted mites on individual hosts were not normally distributed. Spearman rank correlations were used to describe the relationships between parasite prevalence, parasite intensity, resistance prevalence, and resistance intensity. Statistical tests were conducted using SPSS software (IBM Corp. 2012).

Examining the Effects of Parasitism and Resistance

We examined the potential effects of parasitism two ways, on a strongly resistant (*A. fumipennis*) and a weakly resistant (*P. longipennis*) species. First, we tested the hypothesis that parasitism disrupts development and causes body asymmetry. We computed percent forewing asymmetry for each individual using this equation, adapted from Leung and Forbes (1997):

$$\text{Percent asymmetry} = |\overline{X}_{\text{LFW}} - \overline{X}_{\text{RFW}}| / \overline{X}_{\text{all}},$$

where "$\overline{X}_{\text{LFW}}$" = the mean of the three left forewing measurements, "$\overline{X}_{\text{RFW}}$" = the mean of the three right forewing measurements, and "$\overline{X}_{\text{all}}$" = the mean of all six forewing measurements, combined. Data were not normally distributed, so nonparametric Mann–Whitney *U* tests were used to compare the percent asymmetry of individuals with mites to those without mites, for *A. fumipennis* males, *A. fumipennis* females, and *Pa. longipennis* males (only one female *Pa. longipennis* was captured). We used one-tailed Spearman rank correlations to test for significant positive correlations between parasite intensity (number of mites present on parasitized individuals) and percent asymmetry for *Pa. longipennis* males and *A. fumipennis* males and females (analyzed separately).

Second, we conducted laboratory experiments to describe the melanotic encapsulation response of these two species. Methods followed Nagel et al. (2011). Adult *A. fumipennis* and *Pa. longipennis* were collected from Scott's Pond and Swan Lake on the campus of Furman University. Individuals were identified to species and sex and were placed in 1-liter holding cups with a wooden perch and a mesh lid from capture through the course of the experiment. To prevent desiccation, the cups were covered with a moist towel. In the laboratory, 20 lb nylon monofilament fishing line was roughened with sandpaper, cleaned, sterilized with ethanol, and cut into 2 mm segments. A segment of sterile monofilament was inserted into the mesepisternum of each individual to a depth of 1 mm (Nagel et al. 2011). After 24 h, the monofilament was removed and photographed under a dissecting microscope with a Canon EOS Rebel Xs DSLR camera. Photographs were analyzed using ImageJ software (Rasband 2015). For each monofilament segment, six grayscale values were recorded: three measurements were taken on the portion of the segment that remained outside the body ("control"), and three measurements were recorded for the portion of the segment that had been inserted into the thorax ("experimental") and available to

Table 1. The distribution of *Arrenurus* parasitism and resistance for odonates from 11 sites in Greenville and Pickens Counties, SC

Family/species	*N*	Parasite prevalence	Parasite intensity		Resistance prevalence	Resistance intensity	
			X + 1 SD	*N*		X + 1 SD	*N*
Coenagrionidae							
Argia apicalis (Say)	4	25.0	4.00	1	0.0		
Argia fumipennis (Burmeister)	272	20.2	3.35 ± 3.07	55	90.9	98.20 ± 8.97	50
Argia moesta (Hagen)	32	0.0					
Enallagma aspersum (Hagen)	47	25.5	16.75 ± 16.97	12	0.0		
Enallagma civile (Hagen)	2	50.0	17.00	1	0.0		
Enallagma daeckii (Calvert)	16	18.8	1.67 ± 1.16	3	33.3	100.00	1
Enallagma divagans Selys	56	19.6	44.45 ± 118.27	11	9.1	100.00	1
Enallagma exsulans (Hagen)	6	16.7	2.00	1			
Enallagma geminatum Kellicott	34	14.7	2.00 ± 1.41	5	40.0	100.00 ± 0.00	2
Enallagma signatum (Hagen)	38	26.3	18.67 ± 18.44	10	0.0		
Enallagma traviatum (Selys)	119	11.8	3.85 ± 5.73	14	50.0	90.48 ± 16.27	7
Ischnura hastata (Say)	10	10.0	2.00	1	0.0		
Ischnura posita (Hagen)	167	13.2	4.19 ± 3.91	22	18.2	83.33 ± 33.33	4
Gomphidae							
Gomphus exilis Selys	55	0.0					
Libellulidae							
Celithemis elisa (Hagen)	177	26.0	20.26 ± 29.10	46	21.7	96.67 ± 10.54	10
Celithemis fasciata Kirby	113	38.1	15.84 ± 27.64	43	69.8	89.73 ± 24.52	30
Celithemis ornata (Rambur)	33	0.0					
Celithemis verna Pritchard	15	13.3	1.00 ± 0.00	2	50.0	100.00	1
Erythemis simplicicollis (Say)	133	26.3	39.71 ± 89.66	35	20.0	85.79 ± 37.58	7
Ladona deplanata (Rambur)	21	14.3	16.33 ± 24.85	3	0.0		
Libellula cyanea F.	51	5.9	2.67 ± 1.16	3	0.0		
Libellula incesta Hagen	198	14.6	16.29 ± 35.67	29	17.2	70.50 ± 41.55	5
Libellula luctuosa Burmeister	31	29.0	9.22 ± 10.86	9	11.1	100.00	1
Pachydiplax longipennis (Burmeister)	433	17.6	53.92 ± 112.75	76	13.2	57.22 ± 44.80	10
Perithemis tenera (Say)	37	0.0					
Perithemis lydia (Drury)	35	0.0					
Tramea carolina L.	44	9.1	18.50 ± 30.36	4	0.0		

Only species that had at least one mite, or were sampled >30 times, are included. Parasite prevalence ("Prev."): the percentage of individuals that carried at least one mite. Parasite intensity: the mean (±1 SD) number of *Arrenurus* mites on parasitized individuals (*N*). Resistance prevalence ("Prev."): the percentage of parasitized individuals with at least one resisted mite. Resistance intensity: the mean percentage (±1 SD) of resisted mites on individuals that resisted at least one mite (*N*). (Parasite prevalence and mean parasite intensity reprinted with permission from Worthen and Turner (2015).)

melanotic encapsulation. Standardized differences between "control" and "experimental" sections were calculated as:

$$\text{Standardized difference} = (\overline{X}_{\text{exp}} - \overline{X}_{\text{control}})/\overline{X}_{\text{all}},$$

where $\overline{X}_{\text{exp}}$ = the mean of grayscale measurements for the experimental end of the segment, $\overline{X}_{\text{control}}$ = the mean of the grayscale measurements for the control end of the segment, and $\overline{X}_{\text{all}}$ = the mean of all six grayscale measurements. Ten *A. fumipennis* died during the 24 h exposure period and were excluded from the analysis. Mean standardized differences, representing the degree of melanotic encapsulation, were compared between species with a Student's *t*-test (arcsin square-root-transformed data, Sokal and Rohlf 1995).

Results

There was a significant difference in parasite prevalence (percentage of individuals parasitized) across parasitized and well-sampled ($N > 30$) species ($\chi^2 = 112.66$, df = 26, $P < 0.001$; Table 1). As reported previously (Worthen and Turner 2015), several well-sampled species, such as *Argia moesta* (Hagen), *Gomphus exilis* Selys, *Celithemis ornata* (Rambur), *Perithemis tenera* (Say), and *Plathemis lydia* (Drury) were not parasitized at all, whereas prevalence in several other well-sampled species exceeded 25% (Table 1). Parasite intensity (number of mites per parasitized individual) also varied significantly between species (Kruskal–Wallis test, $P < 0.0001$, using species with at least 10 parasitized individuals), with *Pa. longipennis*, *Erythemis simplicicollis* (Say), and *Enallagma divagans* Selys having the highest mean parasite intensity values (Table 1). As reported previously (Worthen and Turner 2015), parasite prevalence correlated with mean parasite intensity (Spearman $\rho = 0.419$, $N = 22$, $P = 0.05$) and median parasite intensity (Spearman $\rho = 0.612$, $N = 22$, $P = 0.002$).

There was also a significant difference in resistance prevalence (percentage of parasitized individuals with at least one resisted mite) among the 11 species with at least 10 parasitized individuals ($\chi^2 = 144.36$, df = 10, $P < 0.001$). Resistance prevalence exceeded 60% for *A. fumipennis* and *Celithemis fasciata* Kirby, but *Enallagma aspersum* (Hagen) and *Enallagma signatum* (Hagen) exhibited no resistance ("Resistance prevalence," Table 1). There was also a significant difference in resistance intensity (percentage of resisted mites on individuals that resisted at least one mite; Kruskal–Wallis test, $P < 0.001$) among the eight species with at least three resisting individuals (Table 1). *A. fumipennis* again showed the strongest resistance response (Table 1). Among these eight species, there was a significant positive correlation between resistance prevalence and resistance intensity (Spearman $\rho = 0.905$, $N = 8$, $P = 0.002$). However, species parasitized more often did not exhibit

a stronger resistance response; parasite prevalence was not correlated with either resistance prevalence (Spearman $\rho = 0.021$, $N = 11$, $P > 0.05$) or resistance intensity (Spearman $\rho = 0.238$, $N = 8$, $P > 0.05$).

We also compared these four variables between sexes, for species with adequate sample sizes. We compared parasite prevalence between sexes for the 12 species had at least 10 individuals of each sex: *A. fumipennis*, *A. moesta* (Hagen), *Celithemis elisa* (Hagen), *C. fasciata*, *Enallagma divagans* Selys, *En. signatum*, *Enallagma traviatum* (Selys), *Erythemis simplicicollis* (Say), *G. exilis*, *Ischnura posita* (Hagen), *Pa. longipennis*, and *Libellula incesta* Hagen. *Celithemis elisa* was the only species that exhibited a significant difference between sexes ($\chi^2 = 10.240$, $P < 0.001$); parasite prevalence was greater in females (52.0%) than males (21.7%). Two species exhibited a significant difference between the sexes in parasite intensity: mite loads were greater on females than on males in *Pa. longipennis* (Mann–Whitney U, $P = 0.002$) and *E. simplicicollis* (Mann–Whitney U, $P = 0.038$). Comparisons of resistance between sexes were limited to the four species that had at least 10 individuals with resisted mites: *A. fumipennis*, *C. elisa*, *C. fasciata*, and *Pa. longipennis*. The only species to exhibit significant differences between sexes was *C. fasciata*, where resistance prevalence in males (80.5%) was higher than in females (14.2%; $\chi^2 = 12.202$, $P = 0.001$) and where resistance intensity was also higher in males than females (Mann–Whitney U, $P = 0.002$).

We compared the percent wing asymmetry between odonates with and without mites in two species: one (*A. fumipennis*) that showed a strong resistance response in nature and another (*Pa. longipennis*) which showed a weak resistance response. There was no significant difference in percent wing asymmetry between parasitized and unparasitized males of either *Pa. longipennis* (Fig. 1; Mann–Whitney U, $P = 0.777$) or *A. fumipennis* (Fig. 1; Mann–Whitney U, $P = 0.310$). Curiously, however, unparasitized *A. fumipennis* females had significantly greater percent wing asymmetry than parasitized females (Fig. 1; Mann–Whitney U, $P = 0.047$). Parasite intensity (number of mites on parasitized individuals) was not significantly correlated with percent wing asymmetry for any of the three groups (*A. fumipennis* males: Spearman $\rho = 0.205$, $P > 0.05$, $N = 49$; *A. fumipennis* females: Spearman $\rho = 0.029$, $P > 0.05$, $N = 6$; *Pa. longipennis* males: Spearman $\rho = 0.304$, $P > 0.05$, $N = 8$).

We also compared the degree of melanotic encapsulation between *A. fumipennis* and *Pa. longipennis*. For *A. fumipennis*, the mean difference in grayscale values between experimental halves of the monofilament (exposed to melanotic encapsulation) and control halves (that remained outside the body) was $18.81 \pm 18.93\%$ ($N = 16$). For *Pa. longipennis*, the mean difference between experimental and control halves was $23.85 \pm 25.63\%$ ($N = 57$). These mean melanotic responses do not differ between species (Student's $t = 0.97$, $P > 0.05$; arcsine square-root-transformed data).

Discussion

The first goal of this study was to describe differences in *Arrenurus* spp. parasitism and host resistance and test the hypothesis that resistance prevalence correlates with parasite prevalence. As is typical for such surveys (Baker et al. 2007, Zawal and Buczyński 2013) and as was reported previously for this data set (Worthen and Turner 2015), there were significant differences among species in parasitism prevalence and intensity. For example, five well-sampled ($N > 30$) species had no mites, whereas six well-sampled species had

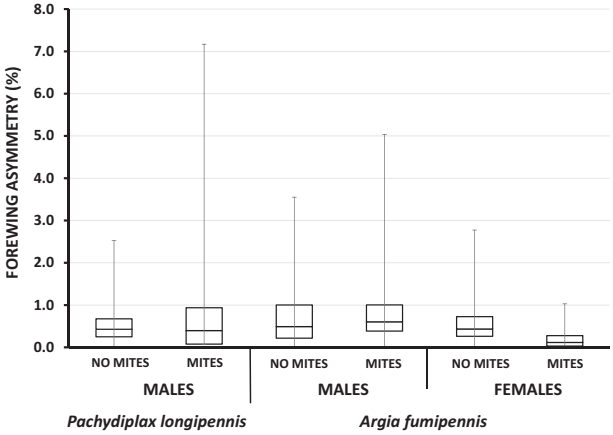

Fig. 1. The percent forewing asymmetry for *Pa. longipennis* (males) and *A. fumipennis* (males and females) that were parasitized ("with mites") or unparasitized ("without mites") by *Arrenurus* spp. water mites. Odonates were collected at 11 sites in Greenville Co. and Pickens Co., SC. Vertical line: range; box defines the first and third quartiles; and horizontal line within the box: median.

prevalence rates that exceeded 25%. These differences were even expressed between congeneric species: *A. moesta* and *C. ornata* had no mites, but prevalence rates were quite high for their respective congeners *A. fumipennis* (20.2%), *C. elisa* (26.0%), and *C. fasciata* (38.1%). Mlynarek et al. (2013) also found significant differences in prevalence rates between closely related congeners.

Prevalence rates in these species were generally consistent with previous studies, with a few specific exceptions. Like Mitchell (1967), we found that *Libellula luctuosa* Burmeister was parasitized frequently (prevalence = 29.0%, second highest among well-sampled species). We also confirmed the host status for *C. elisa* (Mitchell 1959), *En. aspersum* (Mlynarek et al. 2014a), *Enallagma civile* (Hagen) (Conroy and Kuhn 1977), *En. signatum* (Mlynarek et al. 2013), *E. simplicicollis* (Mitchell 1959), *Ischnura hastata* (Say) (Lajeunesse 2007, Lorenzo-Carballa et al. 2011), *I. posita* (Hagen) (Mitchell 1964, Robinson 1983, Botman et al. 2002, Mlynarek et al. 2013), *L. incesta* Hagen (Mitchell 1959), and *Pa. longipennis* (Mitchell 1959). However, although previous studies reported that *Arrenurus* parasitized *Pl. lydia* (Mitchell 1967) and *A. moesta* (Mlynarek et al. 2013), we found no mites on either of these well-sampled species in our study. In addition, although Lajeunesse (2007) found no mites on 53 individuals of *Argia fumipennis atra* Gloyd from two waterbodies in Florida, we found that *A. fumipennis* was one of the most heavily parasitized species in our study, with a prevalence rate of 20.2%. The differences might reflect differences in susceptibility between host populations or subspecies, or differences in host breath of different parasite species occupying different regions.

Differences between sexes were also typical of published patterns. Of the 12 species analyzed, only one (*C. elisa*) showed a sex bias in parasite prevalence (females > males) and only two (*Pa. longipennis* and *E. simplicicollis*) exhibited a significant sex bias in parasite intensity (females > males). These results are consistent with those reviewed by Ilvonen et al. (2015) who concluded that *Arrenurus* prevalence and intensity were similar between sexes in most odonate species. There are exceptions, however, particularly in parasite intensity. Baker et al. (2008) found higher mite intensities for four *Arrenurus* species on *Co. puella*. And although *Lestes disjunctus* showed no difference between sexes in parasite prevalence, females had higher mite intensity in this species, as well (Robb and Forbes 2006). This pattern also occurred in *Lestes dryas* (Yourth

et al. 2001). In *Leucorrhinia intacta* and *L. luctuosa*, however, males had higher intensities than females (Mitchell 1967). These exceptions aside, our results confirm that there is not a sex bias in *Arrenurus* prevalence or intensity in most odonate species.

Resistance to parasitism also varied significantly between species, with *A. fumipennis* exhibiting the greatest resistance prevalence (90%) and resistance intensity (98%). There were also differences in resistance among well-sampled congeners. *En. aspersum* and *En. signatum* were heavily parasitized but showed no resistance; *Enallagma geminatum* Kellicott and *En. traviatum* (Selys) were parasitized at lower rates but exhibited resistance prevalence rates exceeding 40%. Again, these results are not unique. Mlynarek et al. (2014b) found that *Nehalennia irene* (Hagen) exhibited no resistance to *Arrenurus* mites, while the co-occurring congener, *Nehalennia gracilis* Morse, showed complete resistance: resisting every individual mite.

There are several hypotheses to explain differences in parasitism and resistance among host species. For example, if selection favors generalist parasites that exploit wide-ranging and abundant host species, then prevalence should correlate with host range or abundance. This patterns has been documented in the *Arrenurus*—odonate system (Mlynarek et al. 2014a, Worthen and Turner 2015). Likewise, hosts that experience higher rates of parasitism might be more likely to evolve resistance. For example, resistance to *Arrenurus planus* parasitism increased with parasitism prevalence among three *Lestes* species (Yourth et al. 2001). However, resistance might correlate with the diversity of parasite species encountered, not total parasite prevalence. For example, Mlynarek et al. (2014b) suggest that *N. gracilis* could evolve complete resistance because it is a bog specialist and so is probably exposed to a narrower range of *Arrenurus* species than the wide-ranging and susceptible congener, *N. irene*. Contrary to previous studies (Yourth et al. 2001, Nagel et al. 2010), we found no relationship between parasite prevalence and resistance prevalence. However, since we pooled across all *Arrenurus* species, the pattern is potentially confounded by the differences in host niche breadth and parasite species diversity, as suggested by Mlynarek et al. (2014a).

The second goal of this study was to compare the developmental responses to parasitism of a susceptible species and resistant species. *A. fumipennis* mounts a strong resistance response in nature, with a resistance prevalence of 90.2%. In contrast, *Pa. longipennis* was more susceptible to parasites, exhibiting a resistance prevalence of only 13.2%. We hypothesized that *A. fumipennis* would be less affected developmentally than *Pa. longipennis*. There were no differences in forewing asymmetry between parasitized and unparasitized individuals for *Pa. longipennis* males or *A. fumipennis* male or females (analyzed separately), and there were no significant relationships between the degree of asymmetry and parasite intensity. As such, either there is no difference between these species in their developmental response to parasitism, or wing asymmetry is a poor metric. Bonn et al. (1996) showed that the asymmetry of forewing length in *Co. puella* correlated with parasite intensity, but Forbes et al. (2004) found no relationship between wing cell asymmetry and mite load. Given that mites only attach to the adult at metamorphosis and may take 3 h before they form the stylostome and begin to feed, it seems unlikely that parasitism could affect the developmental stability of wing expansion—which is also completed a few hours after emergence. Rather, it seems more likely that positive correlations between asymmetry and mite load (Bonn et al. 1996) are the result of developmentally compromised larvae being more susceptible to parasitism (Forbes and Robb 2008).

Finally, we also compared the responses of *A. fumipennis* and *Pa. longipennis* to a simulated parasitic event: the insertion of a short piece of monofilament line into the thorax (as in Nagel et al. 2011). We hypothesized that the resistant species, *A. fumipennis*, would mount a stronger melanotic response than *Pa. longipennis*. However, there was no difference between species. Nagel et al. (2011) report that encapsulation and resistance are more common in years when parasitism rates are high. It is possible that a low degree of mite emergence, coupled with a stressful lab environment, could cause the low level of melanotic encapsulation in the otherwise resistant *A. fumipennis*. Parasite prevalence rates across species, however, seem consistent with previous studies (Mlynarek et al. 2014a). Perhaps this technique is not suitable for these species, or perhaps *A. fumipennis* is resisting mites through a different mechanism.

In conclusion, there were dramatic differences between odonate species in the prevalence and intensity of parasitism and resistance. There were no relationships, however, between parasite prevalence and the prevalence or intensity of host resistance. Also, there were no differences between the resistant and susceptible species in developmental or immunological responses to parasitism using these methods and metrics.

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