

Selection on mite engorgement size affects mite spacing, host damselfly flight, and host resistance

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ABSTRACT

Question: Are mite dispersion on damselfly hosts, host flight distance, and host resistance related to engorgement size of ectoparasitic larval mites?

Hypothesis: Adaptation to temporary ponds explains why larvae of some mite species engorge to larger sizes, which translates into high costs of parasitism and greater expression of resistance in their hosts.

Organisms: Two species of lepid damselflies and two species of arrenurid water mites.

Field sites: A temporary pond and a permanent marsh in eastern Ontario, Canada.

Methods: We recorded mite prevalence and intensity, and scored spacing of larval mites on newly emerged damselflies and eventual engorgement sizes of mites on mature damselflies from each site. We then estimated the total minimum volume of extracted resources taken by engorged mites of both species. We also measured host flight distance, as well as the magnitude of immune resistance to mites.

Conclusions: Mites from the temporary pond engorged to larger sizes and were more widely spaced than larvae of another mite species from the permanent pond. The total minimum volume of digested host tissues extracted was larger for temporary pond mites, despite them averaging fewer mites per host than the other mite species. Damselflies parasitized by these larger mites were more affected by parasitism based on flight distances. Resistance to these mites occurred more frequently than resistance to mites of the species that engorged much less.

Keywords: costs of parasitism, parasite engorgement, pond permanence, resistance.

INTRODUCTION

Parasites can impose costs on their hosts, including reductions in motor ability, reproductive success, and longevity (Poulin, 2007). However, determining why some parasites are costly to their hosts, whereas others are less costly, is difficult in natural systems where evolutionary traits, and ecological factors, vary spatially and temporally (Sheldon and Verhulst,

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1996). For example, the time that different ectoparasites spend on their hosts can range from minutes to months and might be an important determinant of costs of parasitism, but these costs will likely be mediated also by whether parasites transmit highly virulent pathogens, or whether parasites extract relatively large quantities of resources rapidly from their hosts. One factor that might be important for parasitic water mites is whether they have to obtain enough resources to equip them to withstand drying of ponds following their detachment from hosts, or if they require fewer resources because they detach directly back into water.

It is well known that aquatic organisms that occupy ephemeral water bodies can undergo strong selection to transform quickly in order to adapt to the drying of their habitat. Animals occupying ephemeral ponds often have more rapid growth and/or development rates than conspecifics living in permanent water bodies (Wellborn *et al.*, 1996; Arendt, 1997). The adaptations of many anurans and insects from these ephemeral habitats include phenotypic plasticity and altered life histories (Gomez-Mestre and Buchholz, 2006; Richter-Boix *et al.*, 2006; De Block *et al.*, 2008; Márquez-García *et al.*, 2009). Like their hosts, parasites from ephemeral ponds also might respond to constraints in their environment by taking a lot of resources while in contact with the host. Larval water mites do not have to leave the habitat before it dries, but rather survive the drying period following their detachment (and until the pond refills). Taking more resources from hosts might involve extracting host resources at more rapid rates and/or extracting resources for longer durations. Either way, adaptation of a parasite to an ephemeral environment should impose high costs on hosts as a result.

Larval water mites have been shown to reduce survival and reproduction in odonates (Mitchell, 1967; summarized in Forbes and Robb, 2008). However, the degree to which these parasites affect their hosts is highly variable across species associations. Similarly, resistance of hosts to mites also is variable. The main defensive response of insect hosts is melanotic encapsulation of mite feeding tubes, which kills the mite (Smith, 1988). Maintaining this kind of immune defence is generally costly for hosts, and therefore should not be used unless the costs of parasitism are high (Zuk and Stoehr, 2002; Sandland and Minchella, 2003).

We hypothesized that if pond permanence was an important constraint in water mite life histories, it might influence mite engorgement size and thus the total amount of resources extracted from hosts by mites. This engorgement, in turn, should influence spacing of mites and, depending on intensities of parasitism, reflect the energetic costs imposed by mites on damselflies, and thereby influence mite resistance. The mite, *Arrenurus planus*, is thought to be adapted to ephemeral ponds while the congeneric mite, *Arrenurus pollictus*, inhabits permanent water bodies. *Arrenurus planus* engorge to large sizes by maximizing the amount of host resources extracted (Münchberg, 1952, 1960), which is seen as an adaptation for withstanding periods of pond drying (Wiggins *et al.*, 1980 and as discussed above). Mite spacing was measured to determine if larval mites of each species space themselves to account for the different engorgement sizes that they attain. We measured mite prevalence and intensity (*sensu stricto* Bush *et al.*, 1997), mite spacing and size on congeneric, newly emerged damselflies at a temporary site, and at a nearby permanent marsh site. We then measured engorged mite size on mature hosts at these two sites and estimated the minimum volume of digested host tissues extracted. We also conducted field trials at each site to determine if the distance flown by damselflies differed depending on whether they were parasitized naturally by high or low numbers of mites. We also compared damselfly populations in their likelihood of showing resistance to mites.

METHODS

Study sites and relevant natural history of water mites and host damselflies

Both study sites were located near the Queen's University Biology Station in eastern Ontario, Canada (45°37'N, 76°13'W). Barb's Marsh is a one-hectare marsh surrounded by hay fields and mixed woods. The mite *Arrenurus pollictus* is specific to the damselfly *Lestes disjunctus* at this site. Yzerinac's Pond is a small (<0.1 ha), ephemeral wetland located in deciduous forest about 5 km from Barb's Marsh. This pond has been flooded by beavers (*Castor canadensis*) in recent years, but its margins dry in summer. These conditions are commonly used by the mite *A. planus* (Wiggins *et al.*, 1980). At Yzerinac's Pond, *A. planus* parasitizes several *Lestes* spp. damselflies, as well as several small *Sympetrum* spp. dragonflies (that are also temporary water specialists). No other mite species have been observed on either lestad host at these sites. These hosts are the most common lestad damselflies at the respective sites and have similar morphologies, behaviours, and life histories (De Block *et al.*, 2008).

Larval water mites are phoretic on the final aquatic larval instars of many aquatic insects. When the insects eclose, the mites pierce the host cuticle and form a blind-ended feeding tube (Smith, 1988). *Arrenurus planus* and *A. pollictus* typically attach to the thorax of lestad damselflies, and cannot form another feeding tube once feeding begins. After a pre-reproductive period of about 12 days, female lestids lay multiple clutches of eggs at intervals of 1–5 days (Corbet, 1999). It is during damselfly mate guarding and oviposition that fully engorged mites drop off their hosts (leaving a scar). Detachment from hosts appears to be synchronized, as damselflies at our sites are rarely found with both engorged mites and scars. Engorged larval mites usually then go through predatory deutonymphal and adult stages in water. Most water mite species spend all their time, except for the approximately 2 week parasitic phase, in aquatic habitats.

Arrenurus planus, however, can spend several months out of water. *Arrenurus planus* is one of three closely allied species that share a suite of characteristics: all are obligative temporary pool specialists, have a desiccation-resistant protonymphal stage, and exhibit extreme engorgement. A drought-resistant protonymph allows *A. planus* to overwinter on land (Wiggins *et al.*, 1980). The thick integument in this stage appears to be an adaptation for life in temporary ponds, as most other species of the genus *Arrenurus* inhabit permanent waters and have thin integuments (Wiggins *et al.*, 1980). *Arrenurus planus* do not emerge as deutonymphs until spring meltwaters fill the pools. Previous to this stage, the mites must desiccate and experience cold temperatures before the deutonymph will emerge in the next spring. Large volumes of host resources extracted before detaching also might allow for the shortened deutonymphal stage (Münchberg, 1952; Lanciani, 1971). The adaptations found within the *A. planus* species group are for living in vernal temporary pools (which are inundated with water only in the spring, and are therefore even a more challenging habitat than other temporary pools). Some *Arrenurus* species in northeastern North America (not parasitic on odonates) lack these adaptations and are found in autumnal temporary pools (B. Smith, personal communication). Most of these species overwinter as adults. Females typically store sperm from the previous season, and lay eggs that develop into parasitic larvae in the spring. Although not discussed in depth here, an important constraint for *A. pollictus* mites is the need to match their life cycle to that of their lestad hosts, who typically emerge only during a 7–12 day period each summer. As mentioned, compared with *A. planus*, *A. pollictus* is rarely

found on other species. This might influence the actual numbers of both species of mites found on the different host species.

Damselfly resistance to mites is through melanotic encapsulation of mite feeding tubes, which usually occurs within 24 h of damselfly emergence. Dead mites are flattened and dark, and are always associated with a melanized feeding tube in these systems (Yourth *et al.*, 2001). Dead mites remain attached during the host's reproductive period. Mites that have engorged and successfully detached from the host leave a scar on the host.

Parasitism at host emergence

Teneral damselflies were caught at Barb's Marsh with 1 m² emergence traps set out over vegetation at the marsh margins on 1 June 2009 and checked daily. At Yzerinac's Pond, hand nets were used during daily one-hour surveys starting on 20 June 2009. We ensured that we had accurately estimated the emergence period by starting our monitoring a week before the earliest date recorded for each species and continuing for several days after the last teneral individuals were caught. To estimate prevalence and intensity of mites, the number attached to each damselfly was recorded in the field using a 20× loupe before release. Each damselfly was marked on one of the hind wings with a green permanent marker (Stanford© Sharpie) to avoid recounting it if captured on subsequent surveys.

A qualitative scale (1–3) was used to assess mite dispersion: a tightly spaced cluster of mites on the damselfly thorax was given a score of 1. A score of 2 indicated that mites were more loosely spaced over the thorax, or that one or more was located away from the main cluster. A score of 3 indicated that no mites were within close contact with each other. To quantify this scale, a haphazard subsample of 20 parasitized teneral hosts from each site with a range of dispersion scores was preserved in ethanol and examined at 6× magnification with a Leica Dialux 22 compound microscope. We measured the distance between all mites on an individual host and calculated the mean. Total length and width of all mites from these hosts were then measured, with the mite in dorsal view. Some mites were removed from hosts with forceps, but most measurements were obtained *in situ*. The number of mites measured on each host was sometimes lower than the actual mite load because mites were occasionally destroyed during the removal process or were so tightly spaced that accurate measurements were not possible. The qualitative mite dispersion scores in the field at both sites were calculated (using the subsample of 261 mites from 40 hosts) as: 1 = 0.05 ± 0.01 mm ($\pm$ S.D.) apart, 2 = 0.26 ± 0.02 mm, and 3 = 0.69 ± 0.08 mm.

Parasitism on mature hosts

Adult damselflies were netted in a one-hour survey using hand nets at least every other day at each site to estimate prevalence, intensity, and engorgement size of live mites and to estimate host resistance (measured as percentage of damselflies with dead mites). At Barb's Marsh, three observers surveyed damselflies from 3 July to 7 August 2009. At Yzerinac's Pond, one observer surveyed from 8 July to 11 August. We ensured that we had accurately estimated the reproductive period of damselflies at each site by continuing surveys for several days after the last damselflies with fully engorged mites were seen. Within 1 min of capture, we recorded the number of live engorged mites. We then recorded the host's sex, as well as number of scars and dead mites before marking and releasing the damselflies. The length of the damselfly right forewing (distance between nodus and tip) was measured with

digital calipers (± 0.01 mm). For intensity, mature damselflies with mite scars were excluded from analyses because not all scars can be easily seen as damselflies age.

Engorgement size of mites was scored on damselflies (excluding those with dead mites) using a 20 $\times$ loupe and a qualitative scale of 0–3. Mites scored 0 (no engorgement) if their body was flat with no obvious separation between ventral sclerites and the dorsal plate, and legs were completely visible; a score of 1 was given if mites were approximately the length of their dorsal plate, there was a slight separation between ventral sclerites and the dorsal plate, and legs were mostly visible; 2 = mites were twice the length of their dorsal plate and legs partially visible; 3 = mites were fully engorged, or three times the length of their dorsal plate with no legs visible. At Yzerinac's Pond, a score of 4 was given to any mites that were four or more times the length of their dorsal plate. Any mites that had failed noticeably to engorge in comparison with the rest of the mites on the host were noted, and these hosts were omitted from the final analyses ($n = 6$). A haphazard subsample of damselflies was preserved in alcohol during the week of 1–7 August 2009 to measure the size of engorged mites. We measured length and width of all of the mites on 20 damselflies from each site (as described above). The engorgement scores given to mites on hosts in the field at both sites were determined to correspond with the following length measurements (using the subsample of 285 mites from 40 hosts): 0 = 0.29 ± 0.02 mm ($\pm$ s.d.), 1 = 0.36 ± 0.02 mm, 2 = 0.58 ± 0.03 mm, 3 = 0.7 ± 0.09 mm, 4 > 0.7 mm.

Mite volume in cubic millimetres was calculated using the formula $4/3 \pi r^3$. This formula is the same for objects that are spherical (engorged *A. planus*) or prolate spheroids (engorged *A. pollictus*). The width and thickness of the mite body was assumed to be the same along the length. We assumed that mites do not excrete fluids during feeding, unlike members of the suborder Ixodida (Evans, 1992). However, the engorgement size of mites does not necessarily equal the volume of fluids taken from the host because of losses due to assimilation and respiration. We assume here that these rates are similar between mite species. Estimates of individual mite volume were multiplied by the mean intensity at each site to determine the average minimal volume of resources (digested tissues) extracted from parasitized hosts.

We assessed hosts' resistance in each population by recording the number of individuals sampled during the surveys that had one or more dead mites on them and dividing this by the number of parasitized individuals surveyed.

Host flight distance

Trials were conducted between 11.00 and 14.00 h from 10 to 13 July 2009 at Barb's Marsh and from 22 July to 3 August 2009 at Yzerinac's Pond. Mean hourly air temperatures during these periods were collected with instruments housed in a Stevenson screen. At Barb's Marsh, the average temperature was 24.8°C (range 24.5–26.3°C); at Yzerinac's Pond, the average was 25.9°C (range 24.4–30.6°C; i.e. 1.4°C warmer).

Mature male damselflies were netted beside each wetland and used within 2 min of capture. Each damselfly was quickly examined with a 20 $\times$ loupe to count mites (if present) and to estimate their engorgement size. Of those damselflies that were parasitized, only hosts with fully engorged mites and no dead mites were used. Damselflies were held gently by their wings for 20 s approximately 50 cm above the ground. The observer stood at a marked position, within the 0.2 ha area where the damselflies were captured. The closest erect vegetation was ~130 cm (north and south) and ~80 cm (east and west) away. At release,

one or two additional observers watched the flight path of the damselfly. Trials were not conducted under windy conditions ($> 5 \text{ km} \cdot \text{h}^{-1}$). The distance flown from the release point to the first place that the focal damselfly alighted was measured. The focal damselflies were caught, their mites recounted, and their wing length measured. Focal damselflies were marked (as described above) before release.

Statistical analyses

Logarithmic transformations of damselfly wing length and mite intensity were done to satisfy assumptions of normality. We did not limit analyses of engorgement score, or total volume, to hosts of one sex or account for the total mite number on a host because Robb and Forbes (2005b) found no effect of these factors on engorgement size. For mite length and width, data were $\log_{10}$ -transformed and independent *t*-tests were used to compare these measures between damselflies from each site. However, actual values were used to calculate parasite volume. For the flight distance trials, damselflies were grouped as having either high (≥ 10) or low (0–9) parasite loads. Those with scars were classified as low since the experiment was meant to determine the effects of the current mite load on flight distance (in addition, very high prevalence meant that unparasitized males were rare). Wilcoxon two-sample tests were used to compare the distance flown by damselflies between groups. Differences in prevalence and resistance between sites were determined using Clopper-Pearson estimates of 95% confidence levels. Differences in intensity between sites were compared using *t*-tests. Analyses were completed in JMP (SAS Institute, 2009).

RESULTS

Parasitism at host emergence

Emergence of damselflies at Barb's Marsh began on 25 June 2009 and lasted for 9 days. The prevalence of unengorged mites was 100% ($n = 40$ hosts), and intensity ($\pm \text{s.e.}$) was 21.6 ± 1.6 mites per parasitized host (Table 1). There was no sex bias in any of these measures. The average mite dispersion score given in the field was 1.5. The mean ($\pm \text{s.d.}$) distance between

Table 1. Summary of data collected on unengorged mites at host emergence at both sites (BM = Barb's Marsh: mite = *Arrenurus pollictus*, damselfly = *Lestes disjunctus*; YP = Yzerinac's Pond: mite = *Arrenurus planus*, damselfly = *Lestes forcipatus*)

Site	<i>N</i>	Prevalence (%)	Intensity	Dispersion	<i>N</i>	Mite measurements		
						Length (mm)	Width (mm)	Spacing (mm)
BM	40	100	21.6 ± 1.6	1.5	125	0.31 ± 0.04	0.23 ± 0.03	0.13 ± 0.10
YP	30	80	12.9 ± 1.2	2.4	136	0.30 ± 0.03	0.24 ± 0.04	0.41 ± 0.26

Note: *N* = sample size of newly emerged damselflies, mite prevalence (%), mite intensity (mean $\pm$ s.e.). Under the heading "Mite measurements": mean dispersion score of mites on all hosts sampled in the field (0–3, see text), sample size of mites measured on a subsample taken from 20 of these hosts from each site: mite length, mite width, and spacing between mites (mean $\pm$ s.d.).

mites on the subsample of 20 hosts ($n = 125$ mites) was 0.13 ± 0.1 mm (range 0–0.7 mm). The mean ($\pm$ s.d.) length of mites was 0.31 ± 0.04 mm and their width 0.23 ± 0.03 mm.

At Yzerinac's Pond, emergence began on 3 July 2009 and continued for 13 days. The prevalence of unengorged mites was 80% ($n = 30$ hosts), and intensity ($\pm$ s.e.) was 12.9 ± 1.2 mites, with an average mite dispersion of 2.4. There was no sex bias in any of these measures. The mean ($\pm$ s.d.) length of mites ($n = 136$) was 0.30 ± 0.03 mm and their width 0.24 ± 0.04 mm. Distance between mites was quite variable (range 0–1.7 mm; mean $\pm$ s.d. = 0.41 ± 0.26 mm).

There was no significant difference between unengorged mites from the two sites in terms of length ($t = -2.17$, d.f. = 254.1, $P = 0.98$) or width ($t = -1.3$, d.f. = 248.7, $P = 0.09$). However, the distance between mites in the subsample from Yzerinac's Pond was much larger than at Barb's Marsh ($t = -15.79$, d.f. = 253.8, $P < 0.00001$). This was also evident in the large difference in dispersion scores recorded in the field (2.4 vs. 1.5). The dispersion scores were not confounded with mite number on a host, as there was no significant relationship between intensity and dispersion score on hosts at each site (Yzerinac's Pond: $r^2 = 0.02$, $\chi^2 = 2.23$, $P = 0.52$; Barb's Marsh: $r^2 = 0.04$, $\chi^2 = 3.15$, $P = 0.61$).

Parasitism on mature hosts

We captured 529 and 76 damselflies during surveys at Barb's Marsh and Yzerinac's Pond respectively. Females were larger than males at both sites, which is typical for lestids in this region (Anholt, 1997). Although females from Barb's Marsh had wing lengths 0.6 mm larger than those from Yzerinac's Pond, males from the two sites were similar in size (Table 2). Intensity differed significantly between sites ($t = 4.67$, d.f. = 98.8, $P < 0.001$).

At Barb's Marsh, 87% of the *Lestes disjunctus* population was parasitized by mites, with a mean intensity of 17 mites per host ($n = 444$; Table 2). Males had an average of two more

Table 2. Summary of features measured in mite and damselfly populations at host maturity (sexes listed separately for some measures) at both sites (BM = Barb's Marsh: mite = *Arrenurus pollictus*, damselfly = *Lestes disjunctus*; YP = Yzerinac's Pond: mite = *Arrenurus planus*, damselfly = *Lestes forcipatus*)

Site	Damselfly measurements					Mite measurements			
	<i>N</i>	Wing length (mm)	Prevalence (%)	Intensity	Resistance (%)	<i>N</i>	Length (mm)	Width (mm)	Volume (mm ³)
BM	529		87.3	17.0 $\pm$ 0.5	<1	155	0.68 $\pm$ 0.2	0.56 $\pm$ 0.2	0.11 $\pm$ 0.08
Female	287	14.6 $\pm$ 0.6	87.1	15.9 $\pm$ 0.7					
Male	242	13.4 $\pm$ 0.6	87.6	18.2 $\pm$ 0.8					
YP	76		93.4	9.9 $\pm$ 0.7	46.1	130	1.45 $\pm$ 0.3	1.36 $\pm$ 0.2	1.56 $\pm$ 0.89
Female	34	14.0 $\pm$ 0.2	85.3	8.9 $\pm$ 1.0					
Male	42	13.4 $\pm$ 0.2	100	10.7 $\pm$ 0.9					

Note: Under the heading "Damselfly measurements": sample size of damselflies, wing length (mean $\pm$ s.e.), prevalence (%), intensity (mean $\pm$ s.e.), and resistance (percentage of hosts sampled with one or more dead mites). Under the heading "Mite measurements": mite length and width (mean $\pm$ s.d.) measured on a subsample of 20 hosts from each site, and estimated volume of each mite (mean $\pm$ s.d.).

mites than females. The number of damselflies sampled with one or more dead mites (evidence of successful resistance) was less than 1% (11 of 462; Clopper-Pearson confidence interval: lower 95% = 0.012, upper = 0.042). The average engorgement score recorded in the field was 2.17. The mean length of engorged mites measured in the subsample was 0.68 mm and the width was 0.56 mm ($n = 155$; Table 2). The mean distance between mites on 20 hosts was 0.03 ± 0.02 mm ($\pm$ s.d.). The mean volume of engorged *A. pollictus* mites in the subsample was 0.11 mm^3 . This represents an engorgement size of approximately 16 times the original size. With an average intensity of 17 mites per parasitized mature host, the average damselfly was estimated to lose at least 2 mm^3 of fluids to mites during the 12–16 days of the parasitic encounter.

At Yzerinac's Pond, prevalence on mature *L. forcipatus* was 93%, and intensity was lower than at Barb's Marsh by an average of seven mites. Parasitized males had one more mite on average than females. Of the parasitized damselflies sampled, 46.1% had one or more dead mites (Clopper-Pearson confidence interval: lower 95% = 0.345, upper = 0.578; resistance levels between sites were significantly different). The average engorgement score recorded in the field was 4. The mean length of the engorged *A. planus* mites measured was 1.45 mm and the width was 1.36 mm ($n = 130$). The mean volume of engorged mites was 1.56 mm^3 . This represents an engorgement size of approximately 150 times the original size. The mean distance between mites on 20 hosts was $0.01 \text{ mm} \pm 0.02$ ($\pm$ s.d.). With an average intensity of 9.9 mites per parasitized host, the average damselfly loses at least 15.4 mm^3 of fluids to mites during the parasitic encounter in this population.

There was a significant difference between mites from the two sites in engorged mite length ($t = -24.1$, d.f. = 281, $P < 0.00001$), width ($t = -25.1$, d.f. = 282.6, $P < 0.00001$), and volume ($t = -18.2$, d.f. = 130.7, $P < 0.00001$), but not in spacing at maturity ($t = 3.54$, d.f. = 213, $P = 0.99$).

Host flight distance

Most damselflies flew for distances of less than 2 m upon release and were captured and re-examined. On nine occasions, the observers lost track of test damselflies because they flew very quickly into dense vegetation; these were discounted from the analyses, after determining that mite intensity did not differ. The mean distance flown by all damselflies tested was significantly different between sites (Barb's Marsh 192.6 cm, Yzerinac's Pond 126.2 cm; $t = 1.98$, d.f. = 87, $P < 0.0001$). There was no difference in wing length between *L. disjunctus* (13.23 ± 0.07 mm) and *L. forcipatus* (13.36 ± 0.07 mm) males used ($t = 0.17$, d.f. = 103.1, $P = 0.91$), and wing length did not differ significantly between any of the groups compared. Males that were unparasitized or had scars (and no mites) at Barb's Marsh flew an average of 187.9 ± 11.8 cm ($n = 19$). Seventeen of 70 males tested had scars and no mites; after omitting these from this analysis, the mean intensity was 16.8 mites (range 1–47). Damselflies in the low group (intensity = 0–9 mites) had a mean of 3.6 mites, while those in the high group (intensity ≥ 10) had 22.5 mites. There was no significant difference between the distance flown by males from the groups with high and low parasite loads (high intensity 191.6 ± 8.6 cm, low intensity 193.8 ± 9.8 cm; $t = 0.169$, d.f. = 65.8, $P = 0.56$; Fig. 1).

Males that were unparasitized or had scars at Yzerinac's Pond flew an average of 159.8 ± 13.7 cm ($n = 9$); this difference was not statistically significant between sites (Wilcoxon two-sample test, $t = -1.55$, $n = 20$, $P = 0.14$). At Yzerinac's Pond, 8 of 45 test

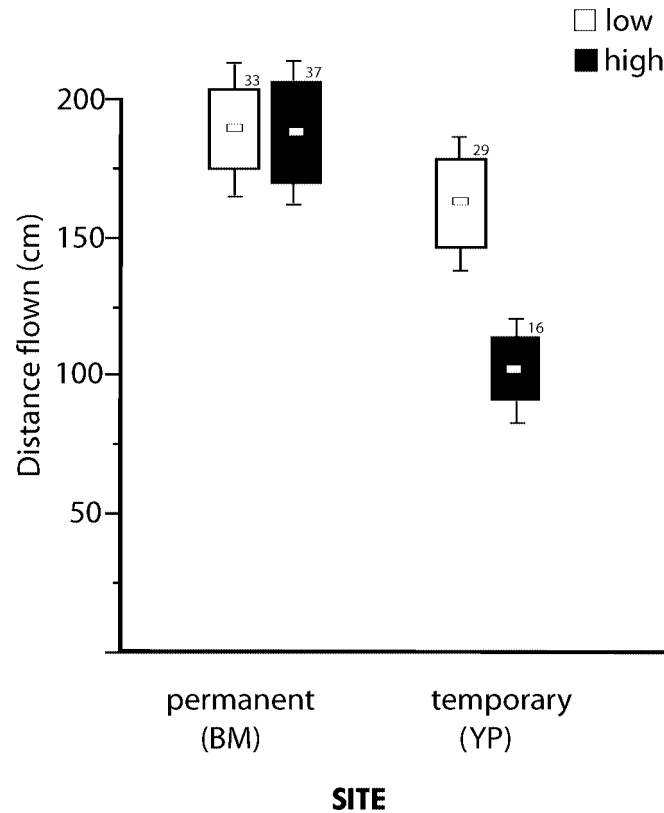


Fig. 1. Mean distance flown by male damselflies parasitized by high (≥ 10) or low (0–9) numbers of mites at each site (BM = Barb's Marsh: mite = *Arrenurus pollictus*, damselfly = *Lestes disjunctus*; YP = Yzerinac's Pond: mite = *Arrenurus planus*, damselfly = *Lestes forcipatus*). Mean is shown as central box, whiskers are $\pm$ s.e., sample size is shown above outer box.

males had scars and no mites. After omitting these, males were parasitized by an average of 8.9 mites (range 1–23). Damselflies in the low intensity group had a mean of 4.5 mites, while those in the high intensity group had 14.7 mites. At Yzerinac's Pond, hosts with high mite loads flew an average of 104.9 ± 8.1 cm, while those with low mite loads flew 166.1 ± 9.2 cm; this difference was statistically significant ($t = 7.89$, d.f. = 41.1, $P < 0.0001$; Fig. 1).

DISCUSSION

Arrenurus planus mites from the ephemeral pond site engorged to about 150 times their original size while *A. pollictus* from the permanent water site only engorged to about 16 times. This size difference is not a result of mites staying on their hosts for longer, as our survey data show that the duration of parasite attachment was similar at each site. Nor are the unengorged ephemeral pond mites simply larger when unengorged, as they were the same size as the permanent water mites when measured at host emergence. Münchberg (1952) noted the marked engorgement in *A. planus*. His estimates were slightly lower (~100 times), possibly because he examined specimens that were not fully engorged. He also showed that

a related mite, *Arrenurus (Brevicaudaturus)* spp. (*sensu* Smit, 1997), engorged to ~1000 times (Münchberg, 1960).

Arrenurus planus probably engorge to large sizes because of adaptations to their ephemeral habitat, which are related to the drought-resistant protonymph instar and brief deutonymph. These mites were more widely spaced on their hosts at host emergence than *A. pollictus* mites were, presumably because they need to have room to accommodate their fully engorged size (mites from both sites were tightly crowded when measured at host maturity). Several studies suggest that crowded mites have reduced fitness (Mitchell, 1967; Lanciani, 1984; Smith, 1988).

Parasite intensity on hosts was much lower at the ephemeral pond. Although prevalence and intensity vary yearly in these populations, these differences are consistent between sites (Yourth *et al.*, 2001; Nagel *et al.*, 2009). Although many factors can contribute to parasite intensity in a population, we suggest that lower intensity at the ephemeral pond site may be related to higher costs of parasitism in the *L. forcipatus/A. planus* system and/or because *A. planus* is a generalist with several alternative host species. Mite intensities may also be lower because of natural selection on mites to reduce host mortality (or to be a direct result of host mortality), since high mite loads presumably reduce the condition of hosts, making them more susceptible to disease or predation. The average parasitized damselfly at the ephemeral pond site loses large quantities of digested tissues (at least 15.4 mm³) during the 2-week parasitic encounter. Few published studies are available to compare with this system, but this amount is much larger than that taken by an individual tick feeding on a mammalian host (Koch and Sauer, 1984). High parasite intensities will also result in increased wing loading, which may interfere with flight ability. If larger mite burdens make hosts (and therefore mites) more vulnerable to predation, then there should be selection on mites to reduce host mortality (Rolff and Martens, 1997). Selection could act on mite behaviour to cause mites to space themselves on hosts during the phoretic phase (where they attend damselfly larvae in the water).

Although we have no direct evidence for higher mortality in damselflies with higher mite loads in this study, the possibility of increased predation on heavily parasitized hosts is suggested by the flight distance trials. Damselflies with high mite loads at Yzerinac's Pond flew for shorter distances than those with low mite loads. A similar pattern was documented in *Nehalennia speciosa* damselflies parasitized by arrenurid mites (Reinhardt, 1996). Of course, factors such as damselfly size, sex, ambient and body temperature, and wing morphology (Bots *et al.*, 2009) will influence flight ability. We attempted to control for some of these factors by conducting trials with only males, over a few days, and by monitoring ambient temperatures to ensure that conditions were similar during all trials. There were also no differences in size (measured as wing length) between groups.

There were no differences in the distance flown by lightly and heavily parasitized damselflies at the permanent water site, which was not the case at the ephemeral site. This suggests that mites from the temporary pond have more serious negative effects on the flight abilities of their hosts. Although other ecological and evolutionary factors could be responsible, we suggest that the pattern may be due to the larger engorgement sizes of the ephemeral pond mites. However, future studies should determine whether there are differences in flight ability between these systems when mites are small. In addition, we do not know if the distance flown in our experiment is an accurate measure of a damselfly's ability to escape predation. Short flights combined with behaviours to make the host more cryptic may actually be a better strategy to avoid some visual predators than a long initial flight.

A final difference between sites was in the immune responses of the damselfly populations. Host resistance to mites has been documented before in these populations, and *L. forcipatus* from Yzerinac's Pond have consistently higher resistance [usually >50% of the parasitized population (Yourth *et al.*, 2002; Robb and Forbes, 2005a, 2005b)] than *L. disjunctus* [< 13% from 2002 to 2008 (Nagel *et al.*, 2010)]. There are several reasons why resistance may be lower at Barb's Marsh. First, the cost of resistance may be much higher here. Since the mean number of mites per parasitized host is higher, the cost of mounting a melanotic encapsulation response against so many feeding tubes may be too high. At Yzerinac's Pond, intensity is lower so there are fewer feeding tubes on a parasitized host to be encapsulated. Second, the cost of parasitism may be lower at Barb's Marsh because the mites take smaller volumes of fluids, resulting in weak selection for resistance. Third, *A. pollictus* mites at Barb's Marsh may somehow avoid detection and therefore often evade the immune response of the host.

To explore some of these hypotheses experimentally, reciprocal transplants need to be carried out in which *L. forcipatus* are infected with *A. pollictus* (and vice versa) to determine if they resist the novel parasite to the same extent as they do *A. planus*. Such experiments would elucidate whether the patterns result from other factors, such as phylogenetic constraints. We stress that the differences between the two systems described here could be attributed to phylogeny and/or ecology, and only further experiments and replication will allow us to understand the relative contributions of each. Since only one wetland of each type was studied and both host and mite species differed between them, it is not possible to conclude that the observed differences are linked to pond permanence. Unfortunately, we have been unable to locate other systems with these mite–damselfly species in our area to replicate our observations. However, previous work with *A. planus* bears on the question of phylogenetic constraints affecting resistance expression. Yourth *et al.* (2001) found that degree of parasitism (measured as prevalence and intensity) was not a strong predictor of resistance expression across four *Lestes* species. One explanation is that phylogeny plays an important role. However, two sibling species, *Lestes dryas* and *Lestes forcipatus*, had the lowest resistance expression and highest resistance expression, respectively. It appears that resistance expression is labile in some systems, and will depend on costs of parasitism, as well as other factors [e.g. timing of emergence (Yourth *et al.*, 2002)].

Although we have not directly measured the costs of parasitism in these damselfly populations (as increased mortality or decreased reproductive success), our results showing differences in host flight behaviours and resistance suggest that the costs of parasitism are higher at the ephemeral pond site. In addition, previous work on the *L. disjunctus*/*A. pollictus* system (Nagel *et al.*, 2009) showed that parasite-mediated mortality might only be expected in some years and then only directed at females (which might account for mature males and females differing somewhat in mite loads).

We expect that we will find other differences between these mites in terms of their stylostome formation and detachment behaviour. We suspect that *A. planus* mites have much larger stylostomes than *A. pollictus* (which might even pierce the host flight musculature). We have observed differences in the likelihood of detachment of the different mites during host predation events. *Arrenurus planus* detach relatively quickly from hosts that are disturbed, while *A. pollictus* are more reluctant to leave their hosts, even while the hosts are being consumed by a predator (unpublished data). We suspect that this is because *A. planus* can withstand drying, while *A. pollictus* need to detach near water to survive to the next stage.

In summary, mites from the ephemeral pond engorged to much larger sizes than those from the permanent water site. Damselflies at this site invested more in resistance and they flew for shorter distances when heavily parasitized. Given that there are differences in the distance flown and in the resistance levels between the ephemeral and permanent water damselflies, we infer that the costs of mite parasitism differ at the two sites. We suggest that this is due to mite engorgement size, which may be shaped by adaptation to ephemeral ponds.

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REFERENCES

- Anholt, B.R. 1997. Sexual size dimorphism and sex-specific survival in adults of the damselfly *Lestes disjunctus*. *Ecol. Entomol.*, **9**: 127–132.
- Arendt, J.D. 1997. Adaptive intrinsic growth rates. *Q. Rev. Biol.*, **72**: 149–177.
- Bots, J., Breuker, C.J., Van Kerkhove, A., Van Dongen, S., De Bruyn, L. and Van Gossum, H. 2009. Variation in flight morphology in a female polymorphic damselfly: intraspecific, intrasexual, and seasonal differences. *Can. J. Zool.*, **87**: 86–94.
- Bush, A.O., Lafferty, K.D., Lotz, J.M. and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *J. Parasitol.*, **83**: 575–583.
- Corbet, P.S. 1999. *Dragonflies: Behavior and Ecology of Odonata*. Ithaca, NY: Cornell University Press.
- De Block, M., McPeck, M.A. and Stoks, R. 2008. Life-history evolution when *Lestes* damselflies invaded vernal ponds. *Evolution*, **62**: 485–493.
- Evans, G.O. 1992. *Principles of Acarology*. Wallingford, UK: CAB International.
- Forbes, M.R. and Robb, T. 2008. Testing hypotheses about parasite-mediated selection using odonate hosts. In *Dragonflies: Model Organisms for Ecological and Evolutionary Research* (A. Córdoba-Aguilar, ed.), pp. 175–188. New York: Oxford University Press.
- Gomez-Mestre, I. and Buchholz, D.R. 2006. Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proc. Natl. Acad. Sci. USA*, **103**: 19021–19026.
- Koch, H.G. and Sauer, J.R. 1984. Quantity of blood ingested by four species of hard ticks (Acari: Ixodidae) fed on domestic dogs. *Ann. Entomol. Soc. Am.*, **77**: 142–146.
- Lanciani, C.A. 1971. Host-related size of parasitic water mites of the genus *Eylais*. *Am. Midl. Nat.*, **85**: 242–247.
- Lanciani, C.A. 1984. Crowding in the parasitic stage of the water mite *Hydrachna virella* (Acari: Hydrachnidae). *J. Parasitol.*, **70**: 270–272.
- Márquez-García, M., Correa-Solis, M., Sallaberry-Ayerza, M. and Méndez-Torres, M.A. 2009. Effects of pond drying on morphological and life-history traits in the anuran *Rhinella spinulosa* (Anura: Bufonidae). *Evol. Ecol. Res.*, **11**: 803–815.
- Mitchell, R.D. 1967. Host exploitation of two closely related water mites. *Evolution*, **21**: 59–75.
- Münchberg, P. 1952. Über Fortpflanzung, Lebensweise und Körperbau von *Arrenurus planus* Marsh., zugleich ein weiterer Beitrag zur Ökologie und Morphologie der im arctogäischen Raum eine libellenparasitische Larvenphase aufweisenden Arrenuri (Ordnung: Hydracarina). *Zoologische Jahrbücher. Abteilung für Systematik, Ökologie und Geographie der Tiere*, **81**: 27–46.

- Münchberg, P. 1960. Erste Mitteilung über die an brasilianischen Odonaten schmarotzenden *Arrenurus*-Larven (Hydrachnellae, Acari). *Archiv für Hydrobiologie*, **56**: 425–440.
- Nagel, L., Robb, T. and Forbes, M.R. 2009. Parasite-mediated selection amidst marked inter-annual variation in mite parasitism and damselfly life history traits. *Ecoscience*, **16**: 265–270.
- Nagel, L., Robb, T. and Forbes, M. R. 2010. Inter-annual variation in prevalence and intensity of mite parasitism relates to the expression of damselfly resistance. *BMC Ecology*, **10**: 5.
- Poulin, R. 2007. *Evolutionary Ecology of Parasites*, 2nd edn. Princeton, NJ: Princeton University Press.
- Reinhardt, K. 1996. Negative effects of *Arrenurus* water mites on the flight distances of the damselfly *Nehalennia speciosa* (Odonata: Coenagrionidae). *Aquatic Insects*, **18**: 233–240.
- Richter-Boix, A., Llorente, G.A. and Montori, A. 2006. A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient. *Evol. Ecol. Res.*, **8**: 1139–1154.
- Robb, T. and Forbes, M.R. 2005a. On understanding seasonal increases in damselfly defence and resistance against ectoparasitic mites. *Ecol. Entomol.*, **30**: 334–341.
- Robb, T. and Forbes, M.R. 2005b. Success of ectoparasites: how important is timing of host contact? *Biol. Lett.*, **1**: 118–120.
- Rolff, J. and Martens, A. 1997. Completing the life cycle: detachment of an aquatic parasite (*Arrenurus cuspidator*, Hydrachnellae) from an aerial host (*Coenagrion puella*, Odonata). *Can. J. Zool.*, **75**: 655–658.
- Sandland, G.J. and Minchella, D.J. 2003. Costs of immune defense: an enigma wrapped in an environmental cloak? *Trends Parasitol.*, **19**: 571–575.
- SAS Institute, Inc. 2009. *JMP*, version 8.0.1. Cary, NC: SAS.
- Sheldon, B. and Verhulst, S. 1996. Ecological immunity: costly parasite defenses and trade-offs in evolutionary ecology. *Trends. Ecol. Evol.*, **1**: 317–321.
- Smit, H. 1997. Australian water mites of the genus *Arrenurus*, with the description of 12 new species, from northern and western Australia (Acari: Hydrachnellae: Arrenuridae). *Records Western Australian Museum*, **18**: 233–261.
- Smith, B.P. 1988. Host–parasite interaction and impact of larval water mites on insects. *Annu. Rev. Entomol.*, **33**: 487–507.
- Wellborn, G., Skelly, D.K. and Werner, E.E. 1996. Mechanisms creating community structure across a freshwater habitat gradient. *Annu. Rev. Ecol. Syst.*, **27**: 337–363.
- Wiggins, G.B., MacKay, R.J. and Smith, I.M. 1980. Evolutionary and ecological strategies of animals in annual temporary pools. *Arch. Hydrobiol. Suppl.*, **58**: 97–206.
- Yourth, C.P., Forbes, M.R. and Smith, B.P. 2001. On understanding variation in immune expression of the damselflies *Lestes* spp. *Can. J. Zool.*, **79**: 815–821.
- Yourth, C.P., Forbes, M.R. and Smith, B.P. 2002. Immune expression in a damselfly is related to time of season, not to fluctuating asymmetry or host size. *Ecol. Entomol.*, **27**: 123–128.
- Zuk, M. and Stoehr, A.M. 2002. Immune defense and host life history. *Am. Nat.*, **160**: S9–S22.

