



# Negative covariance between water mite and gregarine parasitism for adult dragonflies, *Leucorrhinia intacta* (Hagen): an age-related pattern?

Kari M. Kaunisto<sup>1</sup> · André Morrill<sup>2</sup>  · Mark R. Forbes<sup>2</sup>

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## Abstract

Studies on parasite-mediated selection often focus on single parasite taxa infecting single species of hosts. However, host populations experience infections by multiple parasite taxa simultaneously; coinfection is expected to influence how host- and/or parasite-related factors affect host exposure and susceptibility to various parasites, and the resulting patterns of infection. We sampled adult dragonflies from a population of *Leucorrhinia intacta* (Hagen) in eastern Ontario, Canada. Dragonflies were exposed to parasitism by both water mites (Arrenuridae) and gregarines (Eugregarinidae). We tested for covariation between these ecto- and endoparasites, while considering potential sex and age biases in host sampling and patterns of infection. Mite parasitism differed dramatically between host sexes: nearly all collected males were parasitized, whereas only half of females were infested. This was likely due to differences in age distributions between sexes in sampled dragonflies. Water mite and gregarine parasitism showed strong, negative covariation, and coinfection occurred far less often than expected by chance, although these patterns were restricted to samples of females which, unlike male samples, likely included many old and young dragonflies. We report the first observation of negative covariation between internal and external parasite taxa in an anisopteran host and suggest this relationship between water mites and gregarines may be more widespread among Odonata and perhaps other insects than previously surmised. We advance hypotheses based on host age-parasitism relationships as well as variable parasite-mediated selection to help explain the sex specificity of observed coinfection patterns in our samples.

**Keywords** Water mite · *Arrenurus* · Gregarine · Coinfection · *Leucorrhinia* · Dot-tailed whiteface

## Introduction

Often, researchers consider the associations and/or effects of single species of parasites on single species of hosts (Cox 2001; Rigaud et al. 2010). Such studies are tractable and have uncovered a wealth of information on the determinants of both exposure and susceptibility to parasitism, as well as on the fitness costs of parasites and the fitness benefits (to parasites) of parasitizing hosts (Poulin 2007).

However, as noted by Rigaud et al. (2010), parasite species are often generalists attacking more than one species of host (multi-host parasites) and host species and individuals are regularly hosts to more than one species of parasite (multi-parasite host), often simultaneously (i.e., coinfecting individuals). This paper is concerned with the degree of (co)infection of individual male and female dragonfly hosts by two broad taxa of parasites: ectoparasitic *Arrenurus* spp. water mites and Eugregarine gut protozoans.

Many dragonfly and damselfly host species harbor one or both of these two broad taxa of parasites (Forbes and Robb 2008; Ilvonen et al. 2018). An understanding of the parasites' and host's natural history is essential to predicting the degree to which different age or sex categories of dragonfly hosts are expected to be differentially parasitized and ultimately the degree to which hosts are expected to be coinfecting by these different types of parasites. Testing the extent to which hosts are coinfecting under different scenarios (e.g., ages of hosts)

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✉ André Morrill  
andre\_morrill@carleton.ca

<sup>1</sup> Zoological Museum, Biodiversity Unit, University of Turku, 20014 Turku, Finland

<sup>2</sup> Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada

allows researchers to address the degree to which parasites of one species infect hosts independently of other parasite species, versus the degree to which parasites potentially facilitate (or mitigate) coinfection. This is a necessary prelude to studying and understanding the dual impacts of parasites on hosts.

*Arrenurus* (*Arrenurus*) spp. water mites are ectoparasites of adult dragonflies and damselflies, but first enter into a phoretic phase as larval mites are attracted to, and attend to, late-instar larval dragonflies and damselflies (Smith 1988). Once the emerging odonate ecloses, the larval mites abandon the shed exoskeleton of the larval host and attach to the newly emerging adult dragonfly or damselfly. From a position usually on the venter of the host's thorax or abdomen, they pierce the newly formed exoskeleton of the teneral (or prereproductive) adult host and form a feeding tube which allows them to engorge on digested host tissues. Once fully engorged, the larval mite drops off the host when the host is over water for the purposes of defending a territory, mating, or seeking oviposition sites (Smith 1988). The mite then completes its life cycle through two active stages (deutonymph and adult) as predators of aquatic microcrustacea. Except for rare instances (e.g., Robb and Forbes 2006), sex biases in parasitism of odonate hosts is not expected nor observed.

For *Arrenurus* spp. mites, attachment and initial parasitism is simultaneous. Detachment is also more or less simultaneous as infecting mites respond to both external and internal cues (Anderson 2003), providing that larval mites are not resisted (dead mites remain attached to hosts). The majority of mites infesting *Leucorrhinia intacta* (Hagen), the host species in this study, from sites near to and including our site, were *Arrenurus reflexus* with a smaller number of *A. amplus*, based on literally thousands of mites reared from this host species to adulthood (BP Smith, personal communication). *Arrenurus amplus* (< 5% of mites from those samples) were located on the first few abdominal segments and engorge larger than *A. reflexus* which accounted for over 95% of mites and were largely found on abdominal segments 6 through 9. For this reason, hosts either have similarly engorged or engorging mites, no mites, or just dead (remaining) mites. Because of this, the presence of engorging live mites can indicate young adult dragonflies whereas the absence of live mites can include young unparasitized hosts as well as old previously parasitized hosts (which may have an indication of past parasitism such as mite scars; Forbes 1991). In populations where the prevalence of parasitism by mites is 100%, the presence or absence of live engorging mites can be used to age dragonflies (cf. Mitchell 1969).

In comparison to mites, gregarines are trophically transmitted parasites (Bush et al. 2001). For adult dragonflies and damselflies, the gregarine oocysts are ingested along with prey (e.g., dipterans; Åbro 1976) or perhaps imbibed while drinking water (Siva-Jothy 2000). The oocysts excyst once in the midgut and the feeding stage or trophozoite emerges

and attaches to the gut epithelium where it absorbs digesta, grows, and eventually detaches and mates (undergoes fusion or syzygy) while still in the insect's gut. Through a series of reproductive events, oocysts each containing eight sporozoites are produced from each mating of trophozoites and excreted with the feces where they are picked up as debris on legs of dipteran prey. Gregarine parasitism is thought to accumulate with age of the host as more and more food is ingested, with an increased risk of infection mid-season (Forbes et al. 2012). Sex biases in parasitism are expected to the extent that sexes feed differentially or feed on different prey types (paratenic hosts of gregarines) due to, for example, their foraging in different areas or at different times.

Parasite-mediated selection can occur through reductions in, for example, host longevity, fecundity, mating success, or condition. These particular effects of parasitism have been demonstrated in water mite- or gregarine-odonate systems (Forbes and Robb 2008); therefore, selective pressures resulting from water mites and gregarines throughout their coevolutionary history may be important in explaining observed patterns of parasitism when these taxa co-occur.

*L. intacta*, known commonly as the dot-tailed whiteface, is present in all Canadian provinces and its southern limit comprises six states in the USA (from west to east, California to Pennsylvania) and is known to experience high prevalences of *Arrenurus* water mite parasitism (Mitchell 1967; Walker and Corbet 1975). *L. intacta* is one of the most common libellulid dragonflies and has a long flight season (from late May to early August at our study location). Upon emergence, males and females each have dorsal and lateral yellow coloration on abdominal segments 1 through 7. This coloration, except for the characteristic dorsal yellow "dot" on abdominal segment 7, becomes obscured with age in both sexes, though likely at different rates such that only the oldest females have one yellow dot (although sexual dichromatism has been reported; Waltz and Wolf 1988). Males defend territories or patrol the edges of marshes in search of egg-carrying females. After mating, males non-contact guard females which dip their abdomens at the water's surface to lay eggs (Waltz and Wolf 1988).

In this study, we had three primary objectives based on field collections of 285 adult *L. intacta* dragonflies at a single site in Eastern Ontario, Canada. We first addressed whether there were sex or age biases in our samples of dragonflies (assuming a 1:1 ratio of males and females at emergence, cf. Corbet 1999) collected from a series of interconnected hayfields (foraging sites). Dragonflies were not collected at the pond's edge or over the water (territorial or mating sites) and sampling always occurred between the morning and the afternoon. Thus, we expected that territorial males would be underrepresented in samples. We next addressed whether there were sex biases in parasitism by either gregarines or mites and whether such sex biases persisted after controlling

for host age as best we could. We then determined whether mites and gregarines show evidence of inverse relations with one another, as predicted based on different ages being differentially exposed to the two types of parasites.

Importantly, macroparasite distributions often follow a negative binomial distribution (Shaw and Dobson 1995) and combining two negative binomial distributions can produce L-shaped codistributions, with few or no moderate to heavy coinfections (Smith and Cook 1991; Morrill et al. 2017). These emergent codistributions can suggest competitive or even exclusive interactions where there are in fact none; thus, in the interest of understanding parasite covariation separate from the effects of underlying distributions and host-related factors, it is important to ask not just whether significant inverse relations are found but also whether combining the two distributions randomly will produce more or fewer coinfections than observed (cf. Morrill et al. 2014).

## Materials and methods

We collected dragonflies in East field complex or property (44° 32' 29" N, 76° 22' 17" W) under the stewardship of the Queens University Biological Station on five consecutive days (June 8–12, 2018) between 0930 and 1600 hours. Most dragonflies were netted individually and placed individually in 5-mL screw cap plastic vials with a piece of wet paper to avoid drying of the specimen. Vials were then placed within a coolbox within ca. 15 min of capture for transport back to the field station. Once at the field station, dragonflies were frozen at  $-20\text{ }^{\circ}\text{C}$  for transport back to Carleton University for further processing.

Once at Carleton, one of us (KK) confirmed the sex of all 285 dragonflies, performed all enumerations of mites, all dissections for gregarine counts, and all wing length measurements. Evidence of past parasitism by mites (scars, “swollen” terminal abdominal segments) also was recorded. For gregarine counts, guts were first excised from abdomens and split open using a scalpel and viewed using a Zeiss dissecting microscope. Trophozoites, singular and those undergoing fusion or syzygy, were enumerated. Forewing lengths were measured from the distal margin of the first basal wing cell to the nodus. A subsample of 30 males and 31 females were unfrozen, blotted dry and weighed to the nearest 0.0001 g before being processed as described above.

Sex comparisons of wing lengths, mite numbers, and gregarine numbers were based on all 285 collected dragonflies whereas sex comparisons of mass are based on the subsample of 61 dragonflies.

All statistical analyses and randomizations were performed using R, version 3.4.4 (R Development Core Team 2018).

## Results

Females accounted for 198 (or 69.5%) and males for the remaining 87 (or 30.5%) of 285 dragonflies sampled. Given the overall sample size, females are estimated to have accounted for between 63.8 and 74.8% and males for between 25.2 and 36.2% (95% Clopper-Pearson confidence intervals) of *L. intacta* using the interconnected fields as foraging sites. Clearly, our sample of dragonflies was female biased.

Sexes differed in mass with females being heavier on average than males (Table 1; *t* test  $t = -3.84$ ,  $df = 52.9$ ,  $P < 0.001$ ). Males had longer wings on average than females (Table 1; exact Wilcoxon-Mann-Whitney test  $Z = -5.12$ ,  $P < 0.001$ ), with male wing lengths showing negative skew.

Interestingly, about one in every two females sampled had water mites present whereas almost every male carried live water mites (actual prevalence of 46% for females compared to 93% for males; Table 2), a difference that was significant (95% Confidence intervals were non-overlapping; Table 2). Thus, we suspect that young males comprised almost the entire sample of males, whereas the sample of females was made up of many young and many old females. This reasoning is consistent with the fact that our approach precluded sampling of (older) males defending territories over water, and given that large sex biases in *Arrenurus* mite parasitism at the time of host emergence are not expected for *L. intacta* (Mitchell 1967; Smith 1999). Thus, we think that including adult males into our samples would have compensated the observed sex bias in parasitism. Supporting this assertion is the fact that for those individuals carrying live mites (thereby controlling somewhat for host age), the intensity of infection was not different between males and females (confirmed with 1000-replicate bootstrap *t* test; Rózsa et al. 2000). For the subsample of weighed dragonflies, there was an inverse correlation between mass and mite numbers (Kendall’s tau =  $-0.368$ ,  $P < 0.001$ ). However, delving further, this result was explained in that males were more often parasitized and had lower masses than females; there was no relation between mass (plotted in relation to wing length) and intensity of mite parasitism for either sex (Fig. 1). This result is not considered further.

There was no significant difference in prevalence (confirmed using Fisher’s exact test) or intensity of gregarine

**Table 1** Sample sizes and summaries of *Leucorrhinia intacta* body measurements

Sample	Number	Average body mass ( $\pm$ SD)	Average wing length ( $\pm$ SD)
<i>L. intacta</i>	285	124.6 mg ( $\pm$ 19.9)	23.60 mm ( $\pm$ 0.77)
Females	198	133.2 mg ( $\pm$ 20.9)	23.45 mm ( $\pm$ 0.78)
Males	87	115.6 mg ( $\pm$ 14.2)	23.93 mm ( $\pm$ 0.65)

**Table 2** Water mite and gregarine measures of parasitism among *Leucorrhinia intacta* hosts. Prevalence measures are provided with Clopper-Pearson 95% confidence intervals, and mean intensity values with bias-corrected and accelerated bootstrap 95% confidence intervals

Sample	Prevalence (95% CI)	Mean intensity (95% CI)
<i>L. intacta</i> —water mites	0.604 (0.544–0.661)	51.12 (42.32–59.91)
Female hosts	0.460 (0.389–0.532)	45.91 (34.24–57.59)
Male hosts	0.931 (0.856–0.974)	56.98 (46.37–70.54)
<i>L. intacta</i> —gregarines	0.077 (0.049–0.115)	13.77 (8.64–20.67)
Female hosts	0.096 (0.059–0.146)	13.58 (7.80–22.32)
Male hosts	0.034 (0.007–0.097)	15.0 (5.00–21.67)

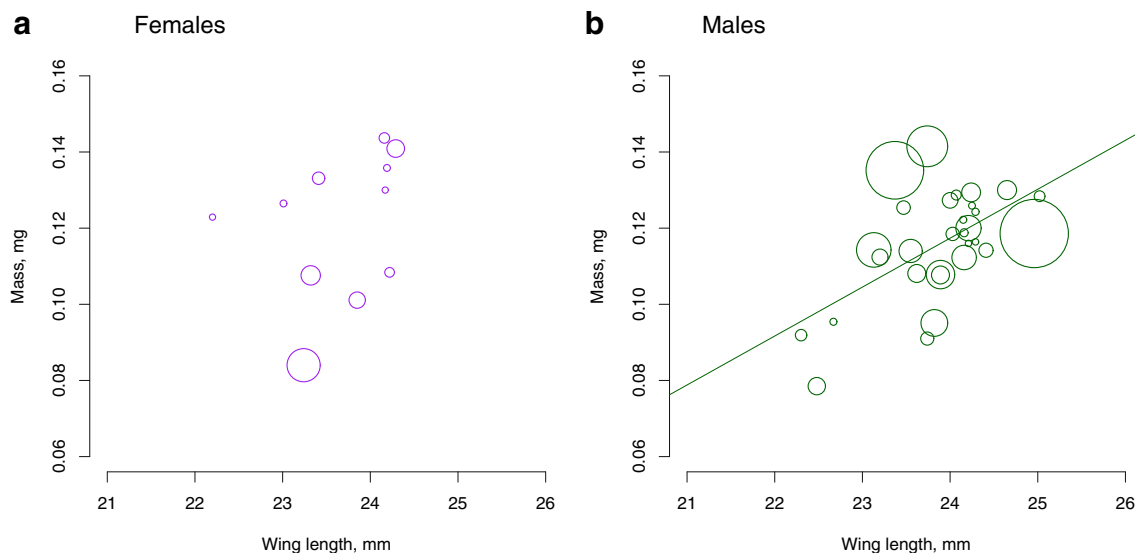
infection between the sexes (Table 2). However, tests comparing mean intensities were not that powerful given only 3 of 87 males and 18 of 198 females were infected with gregarines.

Water mite and gregarine abundances showed significant negative covariation among female hosts ( $\tau = -0.231$ ,  $P < 0.001$ ), although not among males ( $\tau = -0.042$ ,  $P = 0.633$ ), possibly due to the low prevalence of gregarines infecting males, that low prevalence itself possibly resulting from a lack of variation in age within the male host sample. Prevalence of gregarine and water mite co-occurrence within the samples also reflected the female specificity of the covariation: 0.51% of females were infected by both parasite types, while 3.45% of males experienced coinfection; see Fig. 2. Significant negative covariation was also observed among both sexes combined (overall sample:  $\tau = -0.200$ ,  $P < 0.001$ ; 1.4% coinfecting with mites and gregarines). Randomly pairing mite and gregarine abundance data from the entire data set (i.e., removing underlying factors like age)

resulted in coinfection frequencies equal to or lower than the observed value 0.01% of the time ( $N = 10,000$  randomizations), indicating a significantly lower probability than expected of being infected with one of the parasites given an infection by the other, in our samples. This value was similarly 0.01% within the female host sample, but 100% within the male sample (all gregarine-infected males also were infected with water mites in the original sample, i.e., there was no possibility of a higher frequency of coinfection in the randomizations than what was originally observed).

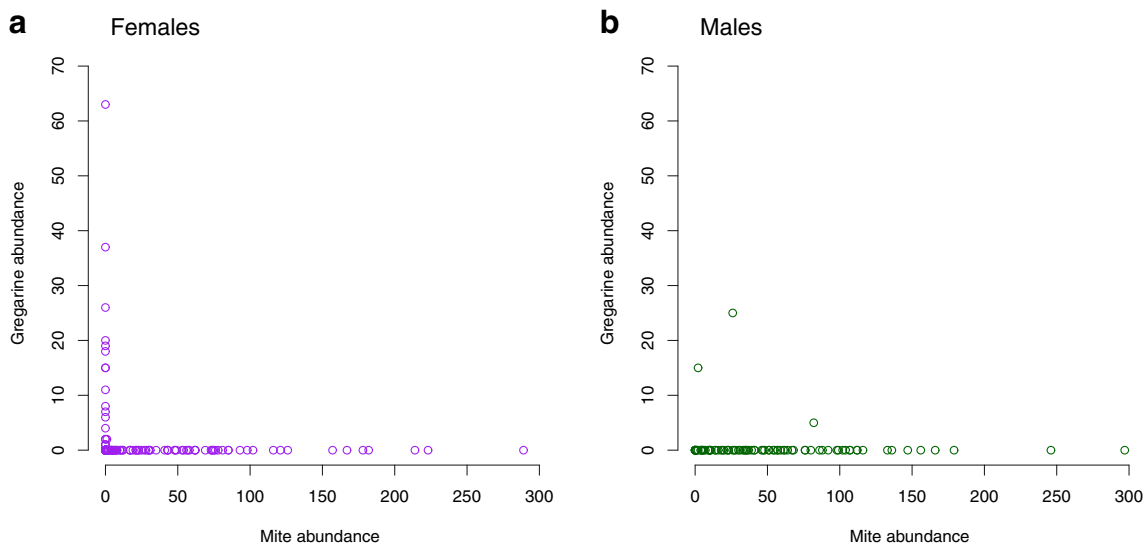
## Discussion

The most salient finding of our study was that coinfections by ectoparasitic mites and gregarines occur infrequently in *L. intacta* dragonflies. That is, young dragonflies still infested with live mites were likely to have few to no gregarines. However, negative covariance between these two parasite taxa was only significant for females and not males (similarly, many fewer coinfections were observed than expected based on randomizations for females, but not for males). That these results were highly significant for females makes us think they reflect a real pattern between these coinfecting taxa, and we suspect that negative covariance between water mites and gregarines may be more widespread among odonates than previously thought, extending to the Anisoptera. Additionally, the trend of negative covariance being restricted to female dragonflies supports the hypothesis that this relationship is due to opposing host age-parasitism relationships between water mites and gregarines as compared to direct or



**Fig. 1** Relationships between *Leucorrhinia intacta* mass and wing length for only those individuals infected with water mites, separated by sex (**a** females, **b** males). Point size reflects relative intensity of mite infection; smallest points indicate intensities of one mite. Positive relationship

between wing length and mass among mite-infected individuals only significant for males (**b**, indicated by solid regression line;  $P < 0.001$ ,  $r^2 = 0.339$ )



**Fig. 2** Codistributions of water mite and gregarine abundances on female (**a**) and male (**b**) *Leucorrhinia intacta*

indirect competitive interactions, as our sampling methods reduced the variance of ages among males while providing what is presumably a wide range of ages among females, given what we know of *L. intacta* life history (Waltz and Wolf 1988).

The major shortcoming of our study was that older males were included rarely in samples (< 7% of males lacked mites and some of those might never have been parasitized). Older males were likely overwater guarding territories and likely had lost their mites. Such males might have acquired gregarines during off-territory foraging bouts and would likely have contributed a “gregarine-only” subset of males to the dataset. The inclusion of this subset might have resulted in significant negative covariance between mites and gregarines for males also.

Only one of the females with mites also had gregarines compared to three of the males. This result also might not be surprising. Only 46% of females had mites suggesting that a lot of the females were indeed old and had lost their mites. There were otherwise 91 young females in the dataset compared to 81 males (1 versus 3 coinfections might then be expected by chance). Alternatively, young males might experience a delay in their loss of mites compared to females (show sexual bimaturism) or acquire gregarines sooner if the pattern identified above is real.

In light of strong, negative covariation between two coinfecting parasite taxa, one must also consider potential hypotheses relating to competitive interactions, either direct or indirect, regardless of any difference in the variances in age between sampled host sexes; these hypotheses need not be mutually exclusive with those relating to host age. Negative covariation between co-occurring parasites contrasts with studies reporting positive relationships between coinfecting ecto- and endoparasites (Holmstad et al. 2008; though see Morrill et al. 2013).

In systems where the establishment or intensities of various parasites is regulated by host energetic investment into immune defenses, decreases in these host resources due to infection by one parasite could lead to increases in subsequent infections by others, and positive covariation between the parasites; in cases where susceptibility-infection relationships are extreme, feedback loops can arise in “vicious circles” of infection (Beldomenico and Begon 2010). However, in cases of negative covariation, particularly when coinfecting taxa occupy two very separate host niches like the ecto- and endoparasites of the present study, other hypothetical indirect interactions relating to either decreases in host susceptibility or changes to host behavior should be considered.

The dragonfly’s melanotic encapsulation response by which mite infection is resisted may become upregulated given the presence or high intensity of ectoparasitic attack, leading in turn to a decrease in successful gregarine establishment in the gut by the same resistance mechanism (but see Siva-Jothy et al. 2001). Alternatively, the energetic, biomechanical, or aerodynamic expenses of water mite parasitism may decrease *L. intacta* foraging efficiency, thereby reducing exposure to gregarine infective stages (Nagel et al. 2010; Morrill et al. 2013). Decreases in mite parasitism due to the presence or abundance of gregarines infecting a host is not expected as gregarines are acquired trophically post-emergence, i.e., after arrenurid water mites are already established. Considering the potential for negative interactions between these two parasite taxa, it could be that opposing host age-parasitism relationships may be evolutionarily advantageous to both parasites by minimizing coinfection and dual costs of parasitism. Negative dual effects might be due to the induction of a stronger immune reaction by cumulative or preexisting parasitism, or by premature

parasite-induced host mortality from combined water mite and gregarine parasitism.

Our results concerning negative correlations between coinfecting water mites and gregarines may seem paradoxical in light of among-species positive associations between these two parasite taxa (their prevalences are positively associated at the odonate host species level; Ilvonen et al. 2018). However, the factor(s) generating among-species positive associations could relate to parasitism more generally (e.g., how widespread and abundant host species are), while within-species inverse correlations, such as in this study, may be explained by parasite taxa showing different age-related patterns or either direct or indirect negative interactions (in a similar vein, host species-level negative covariation between patterns of parasitism need not imply negative covariation between parasites at a within-individual host level). Two things are important to consider in studying this paradox: first, unless age is dealt with explicitly, samples will typically be of mixed ages when documenting within-species patterns; second, there are dangers in inferring within-species mechanisms from among-species patterns. For example, among-species patterns might be used to infer parasites facilitating one another when this need not be the case.

Previous studies have also reported negative covariation between parasite species abundances (Smith and Cook 1991; Honkavaara et al. 2009; for an example wherein there was some controlling for host age among samples, see also Morrill et al. 2013). What is interesting in this case is that females, and likely also males, of this species have to deal with parasitic mites first and then with endoparasitic gregarines later in their adult life. Such results might be illuminated further, but not likely debunked, by better age measures based on mark-recapture data or cuticular sectioning and counting of daily growth rings (Zuk 1987). Although not an original objective of this study, we recorded the number of yellow abdominal segments as a proxy for host age, as these tend to decrease as *L. intacta* mature (Waltz and Wolf 1988). Among females, water mite prevalence significantly increased with increasing numbers of segments with yellow dots, while a significant negative relationship was observed for gregarines, further suggesting opposing host age-parasitism relationships between the two parasite taxa. Clearly, there is a need to explore whether current or past mite parasitism has any further effect on foraging ecology of individuals making individuals more or less susceptible to trophically transmitted parasites such as gregarines. Such investigations are part of an ongoing study.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Åbro A (1976) The mode of gregarine infection in Zygoptera (Odonata). *Zool Scripta* 5:265–275. <https://doi.org/10.1111/j.1463-6409.1976.tb00708.x>
- Anderson TM (2003) Detachment of *Arrenurus hamrumi* larvae (Hydrachnida: Arrenuridae) from *Argia vivida* (Odonata: Coenagrionidae). In: Smith IR (ed) An acarological tribute to David R. Cook (from Yankee Springs to Wheeny Creek). Indira Publishing House, Bloomfield, pp 1–10
- Beldomenico PM, Begon M (2010) Disease spread, susceptibility and infection intensity: vicious circles? *Trends Evol Ecol* 25:21–27. <https://doi.org/10.1016/j.tree.2009.06.015>
- Bush AO, Fernández JC, Esch GW, Seed JR (2001) Parasitism: the diversity and ecology of animal parasites. Cambridge University Press, Cambridge
- Corbet PS (1999) Dragonflies: ecology and behavior of Odonata. Cornell University Press, Ithaca
- Cox FEG (2001) Concomitant infections, parasites and immune responses. *Parasitology* 122:S23–S38. <https://doi.org/10.1017/S003118200001698X>
- Development Core Team R (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Forbes MR (1991) Ectoparasites and mating success of male *Enallagma ebrium* damselflies (Odonata: Coenagrionidae). *Oikos* 60:336–342
- Forbes MR, Robb T (2008) Testing hypotheses about parasite-mediated selection using odonate hosts. In: Córdoba-Aguilar A (ed) Dragonflies and damselflies: model organisms for ecological and evolutionary research. Oxford University Press, Oxford, pp 175–188
- Forbes MR, Mlynarek JJ, Allison J, Hecker KR (2012) Seasonality of gregarine parasitism in the damselfly, *Nehalennia irene*: understanding unimodal patterns. *Parasitology Res* 110:245–250. <https://doi.org/10.1007/s00436-011-2478-1>
- Holmstad PR, Jensen KH, Skorpning A (2008) Ectoparasite intensities are correlated with endoparasite infection loads in willow ptarmigan. *Oikos* 117(4):515–520
- Honkavaara J, Rantala MJ, Suhonen J (2009) Mating status, immune defence, and multi parasite burden in the damselfly *Coenagrion armatum*. *Entomol Exp Appl* 132:165–171. <https://doi.org/10.1111/j.1570-7458.2009.00877.x>
- Ilvonen JJ, Kaunisto KM, Suhonen J (2018) Odonates, gregarines and water mites: why are the same host species infected by both parasites? *Ecol Entomol* 43:591–600. <https://doi.org/10.1111/een.12634>
- Mitchell R (1967) Host exploitation of two closely related water mites. *Evolution* 21:59–75. <https://doi.org/10.1111/j.1558-5646.1967.tb00130.x>
- Mitchell R (1969) The use of parasitic mites to age dragonflies. *Am Midl Nat* 82:359–366. <https://doi.org/10.2307/2423784>
- Morrill A, Mlynarek JJ, Forbes MR (2013) Explaining covariation between endo- and ecto-parasites in spreadwing damselflies (Zygoptera: Lestidae). *Can J Zool* 91:761–765. <https://doi.org/10.1139/cjz-2013-0096>
- Morrill A, Provencher JF, Forbes MR (2014) Testing for dual impacts of contaminants and parasites on hosts: the importance of skew. *Environ Rev* 22:445–456. <https://doi.org/10.1139/er-2014-0026>

- Morrill A, Dargent F, Forbes MR (2017) Explaining parasite aggregation: more than one parasite species at a time. *Int J Parasitol* 47:185–188. <https://doi.org/10.1016/j.ijpara.2016.11.005>
- Nagel L, Zanuttig M, Forbes MR (2010) Selection on mite engorgement size affects mite spacing, host damselfly flight, and host resistance. *Evol Ecol Res* 12:653–665
- Poulin R (2007) *Evolutionary ecology of parasites*. Princeton University Press, Princeton
- Rigaud T, Perrot-Minnot MJ, Brown MJ (2010) Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc R Soc Lond B Biol Sci* 277:3693–3702. <https://doi.org/10.1098/rspb.2010.1163>
- Robb T, Forbes MR (2006) Sex biases in parasitism of newly emerged damselflies. *Ecoscience* 13:1–4. <https://www.jstor.org/stable/42901751>
- Rózsa L, Reiczigel J, Majoros G (2000) Quantifying parasites in samples of hosts. *J Parasitol* 86:228–232. [https://doi.org/10.1645/0022-3395\(2000\)086\[0228:QPISOH\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2000)086[0228:QPISOH]2.0.CO;2)
- Shaw DJ, Dobson AP (1995) Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* 111:S111–S133. <https://doi.org/10.1017/S0031182000075855>
- Siva-Jothy MT (2000) A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proc R Soc Lond B Biol Sci* 267:2523–2527. <https://doi.org/10.1098/rspb.2000.1315>
- Siva-Jothy MT, Tsubaki Y, Hooper RE, Plaistow SJ (2001) Investment in immune function under chronic and acute immune challenge in an insect. *Physiol Entomol* 26:1–5. <https://doi.org/10.1111/j.1365-3032.2001.00206.x>
- Smith BP (1988) Host-parasite interaction and impact of larval water mites on insects. *Annu Rev Entomol* 33:487–507. <https://doi.org/10.1146/annurev.en.33.010188.002415>
- Smith BP (1999) Larval Hydrachnidia and their hosts: biological inference and population structure. In: Needham GR, Mitchell R, Horn DJ, Welbourn WC (eds) *Acarology IX, Symposia*, vol 3. Ohio Biological Survey, Columbus, pp 139–144
- Smith BP, Cook WJ (1991) Negative covariance between larval *Arrenurus* sp. and *Limnochares americana* (Acari: Hydrachnidia) on male *Leucorrhinia frigida* (Odonata: Libellulidae) and its relationship to the host's age. *Can J Zool* 69:226–231. <https://doi.org/10.1139/z91-034>
- Walker EM, Corbet PS (1975) *The Odonata of Canada and Alaska*, vol 3. University of Toronto Press, Toronto
- Waltz EC, Wolf LL (1988) Alternative mating tactics in male white-faced dragonflies (*Leucorrhinia intacta*): plasticity of tactical options and consequences for reproductive success. *Evol Ecol* 2:205–231. <https://doi.org/10.1007/BF02214284>
- Zuk M (1987) Age determination of adult field crickets: methodology and field applications. *Can J Zool* 65:1564–1566. <https://doi.org/10.1139/z87-242>