Large males have fewer water mites (Arrenurus sp.) on the Variable Damselfly (Coenagrion pulchellum)
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Abstract

Ectoparasitic water mites *Arrenurus* sp. (Dugés, 1834) may affect damselflies in different ways resulting in lower longevity and reproduction success. We studied the variation of water mite occurrence on the Variable Damselfly *Coenagrion pulchellum* (Vander Linden, 1823) in relation to the host's sex, location and wing length, and the amount of black pigment on the abdomens of males. In our study we found that water mite prevalence and abundance were higher on females. Location of the populations did not affect the prevalence of water mites, nor did the colouring of males. The prevalence and abundance of water mites was lower on larger males than on smaller ones. Our results suggest that females are likely to have more water mites due to different behaviour and life history strategies. According to our results male body size is a sign of good condition and, thus, of sufficient resources available to be directed to strengthening their immune systems.

Key words: *Coenagrion pulchellum*, Variable damselfly, *Arrenurus* sp., Water mite, *Zygoptera*, sex-bias, body size
Introduction

Good condition of an individual implies a high level of fitness and immunity (Jacot et al. 2004). It has been found that individuals with higher immunity can defend themselves better against parasites (Leung et al. 2001), but usually have a lower reproduction success (Rolff and Siva-Jothy 2003). The condition traits of an individual are measured using several parameters, such as body size (Forbes and Baker 1991), fluctuating asymmetry (Bonn 1996; Rantala et al. 2000) and colouration (Rantala et al. 2000). For example the fluctuating asymmetry of a damselfly species has been connected with the number of water mites on individuals (Bonn et al. 1996). It seems that colouring, can also vary in relation to parasitism. For examples, on the monarch butterfly, Danaus plexippus (Linnaeus, 1758), the amount of melanin in the wings has been found to correlate negatively with the amount of parasites (Lindsey and Alitzer 2009). Also in damselflies it was found that immunity of Calopteryx splendens (Harris, 1780) increased with increasing melanin-based wing spot size (Rantala et al. 2000).

The host parasite relationship of water mites and odonates is widely studied and there are several ways by which mites are shown to affect their hosts (Ilvonen et al. 2016). The occurrence and effects of water mites on odonates varies e.g. among individuals, sexes (Ilvonen et al. 2016), populations (Kaunisto and Suhonen 2013) and species (Ilvonen et al. 2016). It has, for example, been suggested that appearance and body size can have an effect on whether individuals of one sex have more parasites than individuals of the other sex (e.g. Forbes and Baker 1991). In addition larger individuals may have more resources to allocate to their immune systems to defend against parasites.
It is possible that water mite prevalence, abundance and intensity depend on the host's sex. In previous studies it has been found that the prevalence of parasites (the proportion of infected individuals) is higher in female damselflies (e.g. Forbes et al. 2004; Bunker et al. 2013, Ilvonen et al. 2016). However, in other studies male damselflies have been observed to have more parasites (e.g. Robinson 1983; Lajeunesse et al. 2004; Ilvonen et al. 2016). Several explanations for both results have been given. For example, it is possible that females have heavier parasite burdens because they are larger and therefore can provide more resources for parasites (e.g. Nagel et al. 2009). It also has been found that due to different life history strategies, females could have a higher immune system and would, therefore, be better able to defend themselves against the parasites (Joop et al. 2006). Water mites may also prefer female hosts as they are forced to return to the waterbed to lay their eggs, providing parasites with a way to drop themselves back into the water to continue their life cycle (Baker et al. 2008). On the other hand males could have more parasites because they allocate their resources more to pre-reproductive activity, such as mate finding and territorial contests, and therefore result in having lower immunity levels and more parasite as a trade-off (Honkavaara et al. 2009).

Water mites (Arrenurus sp. Dugés, 1834) are parasites commonly found on odonate species (Åbro 1990; Ilvonen et al. 2016) inhabiting nutritious waterbodies. Female water mites lay their eggs in the water. In a couple of weeks the eggs hatch and the newly hatched water mite larvae search for a host, such as an odonate larva. During the host’s last stage of metamorphosis, the microscopically small mites move from the odonate larva onto the adult and rise out of the water together with it. The mite forms a feeding tube through the cuticle of an emerging odonate. The mites generally attach themselves onto the ventral side of odonates, near their legs. Thus the cleaning acts of their host do not reach them (Corbet
1999). Water mite larvae may also attach themselves to the abdomen of the damselfly (Baker et al. 2006) or on its wings (Zawal and Jaskula 2008). Damselflies reach their sexual maturity generally after a couple of days above the water level. After becoming mature they return to the waterbody and the mite larvae drop themselves into the water to continue their life cycles as aquatic predators (Smith 1988).

We used the Variable Damselfly *Coenagrion pulchellum* (Vander Linden, 1823) (Odonata) and ectoparasitic water mites as study objects in this study. The study system is suitable for host-parasite studies because *C. pulchellum* is relatively common in Finland and can be found at many types of waterbodies. During its flying period, it is one of the most abundant damselflies. In northern Europe *C. pulchellum* flies from the end of May to the end of July (Karjalainen 2002). This damselfly species varies in body size and colouring and therefore it may be possible to observe the effect of phenotypic variables on parasite burden. Its body length ranges from 34 to 38 millimetres and the length of its forewings from 19 to 21 millimetres (Dijkstra 2006). The males are mainly blue coloured whereas the females' colouring can be either blue or green. Both females and males have black pigment in patches that vary greatly (Dijkstra 2006).

In this study we observed the prevalence, abundance and intensity of water mites depending on the sex, body size, colouring and population of the variable damselfly *C. pulchellum*. Most previous studies concerning the prevalence, abundance and intensity of water mites on damselflies have studied a single population only. However, in order to observe more general patterns of host-parasite interaction we collected our data from four different populations in southern Finland. We have three study questions: (i) Is the prevalence, abundance or intensity of water mites on the variable damselfly sex-dependent? (ii) Is there a relation between the
body size or colour of the damselfly and the infection of water mites? (iii) Is there variation in infection by water mites among populations as has been observed in its sister species Coenagrion hastulatum (Charpentier, 1825)?

Material and methods

Study areas

Damselflies were collected from four populations located in southern Finland: Kyläsaari (60° 12’ N; 24° 68’ E), Järvelä (60° 27’ N; 22° 22’ E), Rauhalinna (60° 24’ N; 22° 23’ E) and Kuusistonsalmi (60° 24’ N; 22° 27’ E). The Kyläsaari and Rauhalinna populations were by small ponds. The pond at Kyläsaari was surrounded by uncultivated vegetation while cultivated fields surrounded the pond at Rauhalinna. The water body at Järvelä consisted of a mixture of wet reed beds and a small lake. The population at Kuusistonsalmi was located in cultivated fields facing a small bay that is part of the Archipelago of the Baltic Sea. Three of the populations, Järvelä, Rauhalinna and Kuusistonsalmi were located within a distance of 3-5 kilometres of each other. The Kyläsaari population was located about 160 kilometres away from the others.

Collecting of data

The damselflies were collected during the summer of 2013 between the 28th of June and the 2nd of July. Only adult coloured individuals were included in this study. The weather conditions and temperatures were similar during all days of data collection. The individuals were caught while flying around the water body or in nearby vegetation. The individuals
were captured on a strip that followed the shoreline that was 50 metres long and 20 metres wide.

The length of each individual’s right forewing was measured using a digital calliper with a precision on 0.01 millimetres. The wing length was used as a variable to indicate the body size of the damselflies (e.g. Forbes and Baker 1991). The number of water mites attached to the captured damselflies was enumerated with a loupe. During the data collection marks left by mites already detached were not taken into account, which may have resulted in a decrease of the total amount of water mites observed. However, in a previous study carried out with *Coenagrion hastulatum*, a damselfly species closely related to *C. pulchellum*, it was observed that the proportion of scars on damselflies, compared to the mites still attached, is so low that the scars are highly unlikely to impact on the prevalence, intensity or abundance of water mites (Kaunisto and Suhonen 2013).

The amount of black pigment on each individual was estimated from photographed damselflies. The abdomen of each individual was photographed against a white paper to enable the observation of colouration afterwards. Photographs were connected to individuals by giving each damselfly an identification number, which was included bot in the photographs and the field data. Afterwards, individuals were divided into three classes according to the amount of black pigment each individual had on its abdomen (1 = less than 70% of the abdomen is black, 2 = from 70% to 85% of the individual’s abdomen is black, and 3 = more that 85% of the abdomen is black). Before being set free, each individual was marked with coloured paint on the dorsal side of its thorax to ensure that the same individual was not counted again.
Statistical analyses

To estimate the proportion of parasitized individuals in relation to all the collected damselflies, prevalence, i.e. the proportion of infected individuals in a species (Bush et al. 1997) was calculated for both sexes of each host population. Clopper-Pearson 95% confidence intervals were calculated for prevalence (see Zar 1999). Also parasite abundance, i.e. the number of mites on the damselflies (Bush et al. 1997), and its average, mean abundance, were calculated. Based on intensity, i.e. the number of parasites on an infected individual (Bush et al. 1997), the mean intensity, i.e. average number of parasites in infected individuals (Bush et al. 1997), was calculated.

Differences in prevalence between sexes were calculated by using Fisher’s exact test. The variation of water mite mean abundance and mean intensity between males and females was calculated with the Kruskal-Wallis non-parametric ANOVA. The relation between wing length and prevalence was calculated using a binary logistic regression (binomial distribution, logit link function). Infected individuals were marked as 1 and uninfected individuals as 0. The effect of colouring on prevalence in males was estimated using a generalized mixed model (binomial distribution, logit link function) with population as a random factor. The effect of wing length on water mite abundance was estimated using a generalized mixed model (negative binomial distribution, logit link function). All statistical analyses were done with SAS 9.3 statistical analysis software.
Results

Altogether 126 adult variable damselflies were captured from four different populations (Table 1). At the Kyläsaari population 25 males and 3 females were collected. At Järvelä the number of collected damselflies was 31 and 7 respectively. At Rauhalinna population 30 males and 2 females were collected and at Kuusistonsalmi 25 males and 4 females were caught.

*Parasite burden between sexes*

More than 60% of damselflies were not parasitized and only 5% had more than 10 parasites (Figure 1.) The water mites were located on the thorax and abdomen of the host. The overall prevalence of females was higher (75%, Clopper Pearson 95% lower Cl=47.6% and higher Cl=92.7%) than the overall prevalence of males (32%, Clopper Pearson 95% lower Cl=23.9% and higher Cl=42.0%). Water mite prevalence between sexes differed significantly (Fisher-test, $P = 0.0019$, Figure 2).

The abundance of water mites on damselflies varied between 0 and 30 (Figure 1). Water mite abundance on females was 3.9 (SD = 7.1, $n = 16$) and 1.4 (SD = 3.0, $n = 111$) on males. Abundance was significantly higher on females (Kruskal-Wallis Test, $\chi^2_1 = 9.54$, $P = 0.0020$, Figure 3).

The intensity varied on males between 1 and 14 with a mean intensity of 4.2 (SD = 3.9) and on females between 1 and 30 with a mean intensity of 5.2 (SD = 7.7). There was no
significant difference in intensity between sexes (Kruskal-Wallis Test, $\chi^2_1 = 0.012$, $P = 0.913$).

*Water mite prevalence among populations*

The water mite prevalence on damselflies in a population varied between 31% and 43%. Altogether 38% of captured individuals harboured water mites. The Rauhalinna population had the highest prevalence in males (41%). The lowest prevalence in males was at Järvelä (29%). The highest prevalence in females was found in Kyläsaari (100%) and the lowest in Rauhalinna (50%). The location of population did not significantly affect the occurrence of water mites on damselflies in this study (Generalized mixed model, $F_{3, 122} = 0.32$, $P = 0.81$; Table 1).

*Variation in water mite prevalence and abundance among wing length and colouring of males*

The body size data was reduced from 111 males to 103 males because it was not possible to measure the wing lengths of all males. The average forewing length of males was 20.0 millimetres and the wing lengths varied between 18.9 and 21.6 millimetres. The mean abundance of water mites on *C. pulchellum* males decreased when wing length increased (Logistic regression, $\chi^2_1 = 9.38$, $P = 0.0022$; Figure 4). Larger *C. pulchellum* males had a lower water mite abundance than smaller individuals (Generalised mixed model, $F_{1, 101} = 4.52$, $P = 0.036$; Figure 5). The two males that had the highest water mite abundance (14 water mites) were among the smallest individuals captured. The length of their right forewing was less than 19.5 millimetres.
Colouring did not affect significantly the abundance of water mites in variable damselfly males (Generalised mixed model, $F_{2,1} = 1.28, P = 0.529$; Table 2). 10% of male damselflies had less than 70% of their abdomen coloured black. Black pigment covered from 70 to 85% of the abdomen of 66% of males. One quarter (24%) of males had abdomens of which black pigment covered more than 85%. Of the males with least black pigment 9% were infected, of the second lightest 34% were infected, and of the darkest individuals 37% were infected.

Discussion

In this study we found four main results. First of all prevalence and abundance of parasites of *C. pulchellum* damselflies was sex dependent. Females had a higher abundance and prevalence of water mites than males. Secondly within males, larger males harboured fewer water mites than smaller males. Thirdly the colouring of *C. pulchellum* males did not associate with either the abundance or the prevalence of water mites. And fourthly the study site location did not affect water mite abundance or prevalence in this study. We found that females harboured significantly more water mites than males. The overall level of water mite prevalence, as well as water mite abundance, was lower than in some previous studies on odonates (e.g. Andrés and Cordero 2002). However, Ilvonen et al. (2016) found similar water mite prevalence and intensity on *C. pulchellum* as in our study. The relation between the sex of host and abundance of parasites has been under observation in several previous studies (e.g. Robinson 1983; Forbes et al. 2004; Lajeunesse et al. 2004, Bunker et al. 2013; Ilvonen et al. 2016). A possible explanation for the higher prevalence of water mites on the female *C. pulchellum* lies in different kinds of life history strategies (Joop et al. 2006). It could also be that water mite larvae would prefer damselfly females because
they have to return to water reservoir to lay their eggs. This would maximize the chances of
the host returning to the waterbody and where the parasite can fall into the water and continue
its life cycle (Baker et al. 2008). Water mite larvae might recognize the damselfly females by
for example their pheromones. However, this idea needs detailed experimental studies in the
future.

Females tend to concentrate on feeding and ensuring a sufficient level of immunity to ensure
production of eggs and longevity (Joop et al. 2006). On the other hand males concentrate on
searching efficiently for mates and may reduce their immune system as a trade-off
(Honkavaara et al. 2009). It is also possible that the activity level of infected individuals
differs between sexes and heavily infected males may tend to hide in the vegetation instead of
flying in the proximity of the waterbody (Jaenike 1988). The damselflies collected for this
study were all caught flying by the waterbody. The number of females in this study was low.
This can be due to data collection method as individuals were collected near the waterbody. It
has been found in previous studies that males tend to visit the waterbody more frequently
than females (e.g. Garrison and Hafernik 1981; Stocks 2001).

Not all studies are in line with the hypotheses that host's sex would affect parasite prevalence
or abundance. For example a study that also took place in southern Finland on C. pulchellum
discovered a female bias on water mite prevalence (Ilvonen et al. 2016). According to this
study it is possible that water mite prevalence is similar with both sexes when there is
exposure to parasites. Therefore it could be possible that environmental conditions affect
parasitism more than the host's sex (e.g. Zawal and Buczyński, 2013).
Larger males had lower water mite prevalence and abundance than smaller males. There are several not mutually exclusive explanations for this. Firstly, water mites attach themselves often on to the ventral side of the host, where the host cannot reach them. Damselflies have been found ridding themselves of attached mites by brushing (Forbes and Baker 1990). It is possible that larger males can rid themselves of the mites more effectively. Alternatively, the body size of males could also be related to level of immunity. Larger males may have allocated more resources towards strengthening their immunity and therefore their encapsulation response may be more efficient. Ridding themselves of mites is beneficial for males because females have been found to prefer males with a low parasite burden (Honkavaara et al. 2009). Finally, smaller body size of hosts could be the result of intense feeding of parasites on larval odonates, resulting less energy to be allocated into growing. However, this idea needs detailed experimental studies in the future. Damselflies and dragonflies defend themselves against water mites with a so-called encapsulation reaction. The encapsulating response is used for measuring level of immunity (e.g. Rantala et al. 2000). On the damselfly *Coenagrion armatum* (Charpentier, 1840) it has been found that a higher encapsulating response correlates with a lower parasite burden (Honkavaara et al. 2009).

Water mites may have an effect on the reproduction activity and success of males. It has been found that males with a heavy parasite burden mate less than males with fewer parasites (Andrés and Cordero 2002). In addition females may prefer lightly parasitized males (Cordóba-Aguilar 2008; Honkavaara et al. 2009). This suggests that it would be beneficial for males to invest in their immunity levels at the cost of their reproductive efforts (Forbes 1993). Smaller body size of hosts could also be the result of intense feeding of parasites. However, this idea seems to be unlikely because we used only adult damselflies, which had had already
developed to their full body size. The only possibility is that water mites were attached to the individual before its emergence and for that reason the wing length was shorter. However, this idea needs detailed experimental studies in the future.

Phenotypic variance has been found to affect the immunity on damselfly \textit{Calopteryx splendens} (e.g. Rantala et al. 2000). Also it has been found that low genetic variance in individuals is related to high levels of parasitism (Kaunisto et al. 2013). However studies on phenotypic as well as genetic variance in relation to parasitism have generally focused on the abundance of gregarines, i.e. common endoparasitic organisms found on damselflies (e.g. Kaunisto et al. 2013). Alternatively, in this study we have concentrated on ectoparasitic water mites. In the light of the results of this study, it could be suggested that the effect of genetic and phenotypic variance in body size of damselflies on water mite occurrence should be investigated for more details.

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References


Legends for figures

Figure 1. The water mite (*Arrenurus* sp.) abundance on the Variable Damselfly (*Coenagrion pulchellum*).

Figure 2. The water mite (*Arrenurus* sp.) prevalence between sexes on the Variable Damselflies (*Coenagrion pulchellum*). Bars show 95% confidence intervals.

Figure 3. The mean abundance of water mites among female and male Variable Damselfly (*Coenagrion pulchellum*). The bars imply the standard error.

Figure 4. The probability of male Variable Damselflies (*Coenagrion pulchellum*) being infected by water mites in relation with their body size.

Figure 5. The relation between body size and water mite abundance on male Variable Damselflies (*Coenagrion pulchellum*).
Table 1. Prevalence of water mites on the Variable Damselfly (*Coenagrion pulchellum*) on three different sites.

<table>
<thead>
<tr>
<th>Study site</th>
<th>Location</th>
<th>Prevalence</th>
<th>n</th>
<th>Prevalence</th>
<th>n</th>
<th>Prevalence</th>
<th>n</th>
</tr>
</thead>
<tbody>
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<td>36</td>
<td>25</td>
<td>100</td>
<td>3</td>
<td>43</td>
<td>28</td>
</tr>
<tr>
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<td>31</td>
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<td>7</td>
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<td>38</td>
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<tr>
<td>Rauhalinna</td>
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<td>32</td>
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<tr>
<td>Kuusistonsalmi</td>
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<td>25</td>
<td>75</td>
<td>4</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>111</td>
<td>75</td>
<td>16</td>
<td>38</td>
<td>126</td>
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</table>


Table 2. Prevalence of water mites in relation to black pigment of the abdomen of Variable Damselfly (*Coenagrion pulchellum*) males.

<table>
<thead>
<tr>
<th>Proportion of black pigment on abdomen (%)</th>
<th>n (All)</th>
<th>Proportion of samples (%)</th>
<th>n (Infected)</th>
<th>Infected (%)</th>
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<tr>
<td>70-85</td>
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<td>66</td>
<td>26</td>
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<tr>
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<td>24</td>
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