8/10/2006

Bio 390: Evolution

EEB 431: Parasitology

Reproductive success and survivorship of damselfly *Enallagma hageni* infected with ectoparasite *Arrenurus*

By

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KEY WORDS: reproductive success, survivorship, ectoparasites, damselflies

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Abstract

The reproductive success and survivorship of adult male damselflies *Enallagma hageni* infected with ectoparasitic larval water mite *Arrenurus* spp. was examined. Parasitic prevalence/incidence of non-mating vs. mating males was analyzed to determine reproductive success. Body mass of parasite vs. non-parasite infected males was analyzed to determine survivorship. It was discovered that parasites are more prevalent and incident on non-mating vs. mating males. The presence of parasites on male damselflies can significantly depress their mating success. The average body weight of male damselflies with parasites was significantly lower than that of males without parasites only if infected males had high levels of parasites. Thus, parasitic water mites significantly change the survivorship of male damselflies only at high parasite levels. Reproductive success of *E. hageni* is more sensitive to water mite *Arrenurus* than survivorship.

General Biology and Collection of Study Species

Aquatic ectoparasitic larval mites are generalist parasites (Forbes et al. 1999) and have been shown to alter the mating success and survivorship of several species of adult damselflies (Forbes and Baker 1991; Forbes 1991a; Andrés and Cordero 1998; Polak 2003). They have been known to affect survivorship by taking vital nutrition from the damselfly, draining precious body fluid (Andrés and Cordero 1998), or by acting as a vector for transmittable diseases (Polak 2003). Some get onto the damselfly by first attaching to the damselfly larval stage without parasitizing it (Smith 1988; Robb and Forbes 2005a). When the adult damselfly emerges, the ectoparasites reattach and begin to engorge, piercing the host cuticle with a feeding tube (chelicerae) within 24 hours of reattachment (Smith 1988; Forbes 1991b; Robb and Forbes 2005a). Timing is the major factor that influences location of attachment on the host. They usually attach to the first segment they contact (Smith 1988). They then secrete a dense substance to cement them to the host. While attached to the host, the parasite can increase 80 to 90 fold in volume (Smith 1988).

Enallagma hageni is a medium-sized, sexually dimorphic damselfly that is widely distributed across eastern North America (Fincke 1982). It is non-territorial and the flight season occurs from mid-June to early August (Fincke 1982, 1984). Unlike many species of damselflies, females of *Enallagma hageni* cannot be forced to mate. When the male seizes the female, she must raise her abdomen to copulate. If she refuses to do this, she is released within 1-10 minutes (Fincke 1984). Because of the large sex bias and because all females are thought to mate, male mating success is much more variable than female mating success (Fincke 1982).

Mites were chosen because they are readily visible and easy to count on the ventral side of the abdomen and thorax of adult odonates. Adult *Enallagma hageni* are easy to capture with nets whether or not they are in copulation pairs. Because females cannot be forced to mate, direct female choice can be measured to determine male reproductive success. Male reproductive success is highly variable and easy to measure.

Specific Objectives

We hypothesized that mites of the genus *Arrenurus* significantly reduced the survivorship and reproductive success of the male damselfly *Enallagma hageni*. One way to measure fitness is to measure body size (Braune and Rolff 2001). If a histogram of body size for an uninfected population of damselflies follows a normal distribution, we know selection favors an intermediate body size. If the shape, including the mean or variance, of this same curve changes in any way for a population of infected damselflies, all other things being constant, parasites do affect fitness. If parasitic prevalence/incidence on male damselflies is related to whether they can get mates, then the mites do alter the reproductive success of individual damselflies. The objective of the study was to assess natural levels of parasitism and examine the survivorship and mating success of adult damselflies engorged with *Arrenurus*.

Materials and Methods

Study Site

All field data were collected from a stream on the section of Sugar Island that the UM Biological Station oversees. The stream was very shallow (barely knee high) at its deepest point. Less than a half a mile strip of the stream was surveyed. Surveying took place on two days (7/10/06 and 7/17/06). The days were separated by a week which provides a long enough gap for things such as mite density to change considering the briefness of the mating season for *E.hageni* as described above. The weather on both days was fairly sunny and warm.

Data Collection

We captured an abundance of single damselfly males in the population. Towards the end of the last day, an effort was made to only capture copulating damselflies; solitary males were ignored. Units of either single or copulating tandems were placed in individual wax envelopes and were immediately frozen to kill the damselflies. As soon as this was done, the odonates were analyzed using a dissecting scope to observe and to record the parasite data including the number, color and location of the mites *Arrenurus*. Next, the engorged mites were removed from the damselflies with a scraping tool; they could potentially alter the wet mass. Finally, the odonates were weighed on a gram scale that was sensitive to the thousandth of a gram. The wet mass was recorded as well as the specific day each damselfly was collected, the sex, and whether or not the damselfly was captured while copulating. Male damselflies were split into two arbitrary groups based on parasite level: those males that had less than ten parasites were categorized in the low-level parasitism group and those parasites that had ten or more parasites were put in the high-level group.

Statistical Analysis

A comparison was made of the prevalence (frequency of mites with at least one parasite) and incidence (the average amount of parasites per damselfly) for mating males vs. non-mating males and females vs. males. A graph of body weight vs. number of parasites was analyzed to see if there was any relationship between these two variables. Whether prevalence/incidence has a significant effect on the fitness of damselflies was also tested. Finally, parasite prevalence/incidence comparison of the total damselflies between day one and day two to see if the time of mating season has any noticeable trend was conducted. Z-tests, X^2 tests, T-tests and regression tests of significance were done using Microsoft Excel and SPSS.

Results

Of the total sample size of 241 damselflies, 160 were males and 81 were females. All of the females, but three, were captured while copulating with separate males; thus 78 males were mating and 82 males were caught solo. Exactly 60 male and female damselflies contained parasites. A total of 170 damselflies were caught on day one; 71 were caught on day two. The total water mite prevalence was 23.1%. The total water mite incidence was 1.5 mites per adult with a range of 1-45 mites per damselfly.

Host Sex

On average 26.8%±6.6% of males had at least one parasite on them whereas 14.8%±7.9% of females had at least one parasite on them (95% confidence interval, all CI used from hereafter is 95% unless stated otherwise). A chi-square test of homogeneity confirms that these proportions are different from each other ($X^2=4.507$, df=1, $p<.05$).

The average amount of parasites on males (1.743±.853) is greater than the average amount on females (.975±.785). There is not sufficient evidence ($t=1.102$, df=258, $p>.05$) to state that the means are significantly different, thus we cannot conclude that the incidence of parasitism is higher in males than in females.

Timing

On day one, an average of 1.67±.88 mites per damselfly was found while on day two, an average of 1.19±.81 mites per damselfly was found. An independent samples t-test ($t=.710$, df=258, $p>.05$) does not give evidence to state that these two means are significantly different from each other.

The mite proportion of damselflies with at least one mite was .259±.067 on day one and .178±.080 on day two. A chi-square test of homogeneity (X^2=2.169, df=1, p>.05) does not give sufficient evidence to confirm that these population proportions are significantly different from each other.

Reproductive Success

The proportion of mating male damselflies that had parasites (19.2%±8.8, n=78) is significantly less than the proportion of non-mating male damselflies (32.7%±9.1) that had parasites (z=2.021, p<.05, n=101). Thus, the difference in proportions is significantly greater than zero (13.5%±12.7%); there is reasonable evidence to conclude that parasites are more prevalent on non-mating damselflies than mating. A chi-square test of homogeneity (Table 1 for cross-tabulation, X^2=4.03, df=1, p<.05) further reinforces the aforementioned findings.

Table 1 Parasites vs Couple-Mating Cross-tabulation for Male *E. hageni*

		Mites		Total males
		Males with none	Males with at least one	
Couple-mating	Males mating	63	15	78
	Males non-mating	68	33	101
Total males		131	48	179

The average number of mites on non-mating males (.97±.75, n=78) is much less than the average number of mites on mating males (2.34±1.4, n=101) if you include males that have no mites. The means are significant enough to conclude that they are statistically different (t=-1.701, df=150.134, p<.10, CI= 90%).

Survivorship

A regression line was made through the scatter data showing the total number of mites in relation to body weight for all damselflies that had at least one parasite (Figure 1). The equation had the formula y= (-0.0000325) x+0.019. The correlation coefficient (r=-.098) shows that the linear association between the two variables is weak. Furthermore, an F statistic and t-statistic (F=.450, df=1, t=-.671, p>.05) confirm that the slope of the line is not significantly different from zero enough to confirm a strong negative relationship between body mass and the number of parasites.

The average body weight between male damselflies with and without parasites is 19.05±.94 milligrams and 18.96±.64 milligrams respectively. A t-test (t=-.152, df=158, p>.05) concludes that the means are not significantly different from each other which gives us valid evidence to state that parasite prevalence has no effect on body size of male damselflies.

Low-level parasite hosts had a wet mass of 19.1±.541 milligrams and high-level hosts had a mass of 17.3±1.83 mg. Thus the damselflies with high-level parasitism showed a lower mass on average than those of the low-level parasitism group. A t-test shows that the mass averages are significantly different (t=.092, df=158, p<.10, 90% CI). A larger sample size should show an even more significant difference as we used a relatively small sample size, especially in the sample size of high-level parasitism males (n=10). Therefore, although there is reason to believe that parasite prevalence does not have an effect on body size as stated before, there is evidence to conclude that parasite intensity, in terms of high-level parasitism, does have a significant effect on body size.

Discussion

We looked at the effects of the water mite *Arrenurus* on a number of factors of the adult damselfly *Enallagma hageni* as well as the natural characteristics of the host/parasite interaction.

It appears that water mites are more prevalent on males than females, but the incidence levels are not significantly different. Previous studies (Lajeunnesse et al. 2004) suggested that differential habitat use between sexes of some odonates may explain the male bias in parasitism prevalence. Our findings contradict other research (Joop et al. 2006) which states that no difference was found in infection rates of water mites between male and female damselfly morphs. Joop et al. (2006) measured a different species of damselfly, however, and this may be the reason for the discrepancy.

It has been speculated that after the necessary engorgement time, mites of the genus *Arrenurus* use certain cues to know when to detach from the host so they will end up back on a body of water (Smith 1988.) According to reports on the temporal patterns of water mites, as the summer progresses, infestation of mites should be lower (Smith 1988.) Both the prevalence and incidence of mites was not significantly different from each other between the two sampling days that were separated by a week. On day one there were, however, more mites found per damselfly and a larger frequency of damselflies infected with mites. This shows that perhaps separation time of the sampling days were not large enough. In the future, separating the days by more time than a week may give different results.

The results of the present study give good reason to believe that parasites significantly affect the reproductive success of male *E. hageni.* Females may have the ability to detect the bright mites on males, associate this with a weakness, and be more reluctant to mate with them than non-parasitized males. These findings contrast with previous studies (Rolff et al. 2000) where no correlation between water mites and male reproductive success was revealed. One explanation for this conflict is that Rolff et al. (2000) investigated the damselfly *Coenagrion puella* which has a mating system that may differ in important aspects such as population density and territorial behavior.

If females actively detect mites on males and use this characteristic to choose or deny potential mates, then mites directly affect reproductive success. If mites alter the body weight of males and the body weight is the characteristic that the females choose, then mites indirectly have an effect on reproductive success. One could look at the regression line of this study that describes mites vs. body weight and could make the argument that females don't use mites as a factor for choosing mates and that the only reason why they tended to mate with males that had fewer parasites is because only males of high-level parasitism had significantly smaller mass which females don't prefer (Fincke 1982) and this skewed the data. In this case, the parasites only indirectly have an effect on reproductive success. This could be true, but highly unlikely. If females were oblivious to mites when choosing male mates, the effect of choice would be miniscule when dealing with low mite levels just like the effects of body size are with low mite levels. However, the effect is discernable, in fact much more apparent than the change in body size at low parasitism level. Also, the sample size of high-level parasitized damselflies is so small (n=10) that when it was taken out of the sample and the test was redone, there was only a minor change in parasite prevalence of mating vs. non-mating males.

Unexpected for us, the findings give evidence to conclude that survivorship is unaffected by low levels of parasitism. This conflicts with Rolff et al. (2000), who reported that water mites were negatively correlated with body fat and thus reduced host condition. Other than possible sample size differences, the present study may have different results from Rolff et al. (2000) because of differences in how the experiment was conducted. Whereas the present study measured total wet mass, Rolff et al. (2000) may have been able to measure survivorship more accurately through a special technique in which just the body fat content was measured.

The findings of the present study give evidence to conclude that survivorship is significantly affected by high levels of parasitism. This is supported by Leonard et al. (1999) who did work on *Enallagma ebrium*, a very closely related species of *E. hageni.* Perhaps, the male damselfly is able to prevent harm from low levels of mites through some evolved mechanism. Yet the mechanism can only prevent parasite damage to a certain threshold amount of parasites and when the number of mites surpasses that threshold, harm can no longer be prevented. A theory that fits this view is one where that mechanism is in the immune system of the host and there is a trade-off between immune traits and other life history traits (Robb and Forbes 2005b). It is costly to display immune traits and after a certain cutoff amount of parasitism, the cost of immune resistance outweighs the benefit because the life history traits such as hydration or energy become too much depleted.

It is also important to note that *E. hageni* have a teneral phase, a life cycle stage after exclusion from the pupa but before full maturation and coloration. The *Arrenurus* mites are engorged at this stage. Past literature (Rolff et al. 2000) suggests that selection against parasitized individuals occurs at the teneral phase. If this is true, it explains why evidence was found that the water mites had no correlation with body size. Those damselflies that were selected against never made it to the adult stage and thus were never captured. Assuming this theory is correct, a better experiment would have weighed the damselflies during the teneral phase or at early emergence (Braune and Rolff 2001).

A conclusion surprising to us is that the ectoparasites have a different effect on reproductive success of damselflies than on the survivorship of damselflies. We demonstrtate that reproductive success of damselflies seems more sensitive than survivorship because whereas low levels of parasites change the breeding success of male damselflies, only at high levels of parasite incidence does the body weights seem to decrease. These findings are in accord with previous literature (Andrés and Cordero 1998) in that male damselfly mating success is more sensitive to water mite parasitism than survivorship (although this study was done on the damselfly *Ceriagrion tenellum)*. So whereas a female damselfly *E. hageni* can associate even one mite on a male as a cue that the male is not a suitable mate, that same mite is not powerful enough on its own to really put a male's health and survivorship in jeopardy.

Acknowledgements

The authors thank the University of Michigan Biological Station for support. We also acknowledge the summer 2006 evolution class including E. Kay for help in the field. Finally we are grateful for H. Blankespoor for sharing his knowledge about parasitism.

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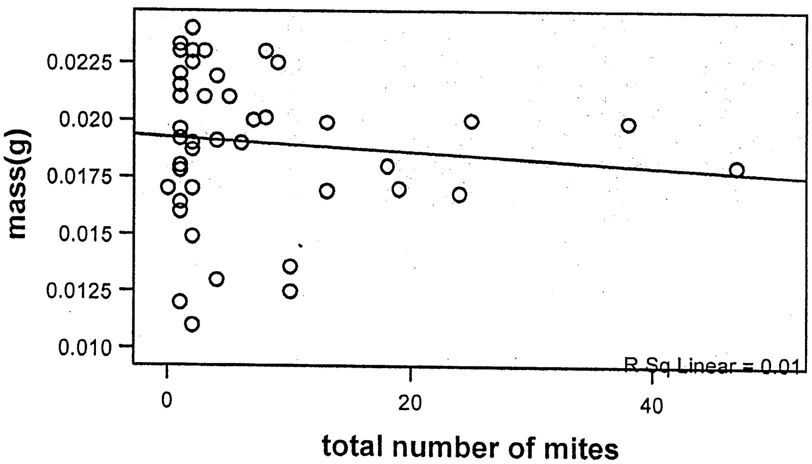
Figure 1. Wet mass in grams against total number of mites of adult male *E. hageni* [mass (g)= (-0.0000325)(#of mites)+0.019].



mass(g)
0.0225
0.020
0.0175
0.015
0.0125
0.010
R Sq Linear = 0.01
0
20
40
total number of mites

Figure 1