

Parasitism of mating and non-mating males and body mass of *Enallagma hageni*

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Keywords: damselfly, parasitism, sexual selection, mass, *Arrenurus*

## **Abstract**

Studies of parasites and how they affect their host reveal intriguing evolutionary aspects about the species studied. Parasitism not only directly affects an individual but can also lead to gradual adaptation in a species. Mating success can be affected by parasitism due to sexual selection and the affect it can have on an individuals ability to mate effectively and produce viable young. The parasitism and mating success of damselflies can be easily studied due to a short mating season and their observable mating style. *Enallagma hageni* is parasitized by the water mite *Arrenurus spp.* beginning at the damselfly larval stage and continuing through the majority of the damselfly mating season, thus the effects the mites have on the mating success *E. hageni* can be readily studied. Body weight can be an indirect indicator of mating success because a smaller body weight can reduce success at male-male competition and attractiveness to females. This study looks at the effects mites have on body weight and the differential parasitism of males in *Enallagma hageni* as a model to further understand how mating success can be affected by parasitism.

Studies have shown that sex may have evolved as a mechanism to resist parasitism (Hamilton 1990). Recombination due to sexual reproduction increases defense by providing a host species with a variety of genotypes, which is especially important for adaptation (Hamilton 1980). As a host evolves, parasites that infect it will co-evolve with it. Parasites and bacteria evolve quickly due to shortened life spans and asexual reproduction (Hamilton 1990), so in order to combat this, sex provides a mechanism for a host to evolve quickly enough to combat the parasitism. Mechanisms that increase mating success such as an increase in body weight would result in an increase in efficiency of host evolution so that the species can be more successful at counteracting parasites.

Numerous studies have demonstrated the effects of the parasite, *Arrenurus spp.*, on various species of adult damselflies (Abro 1992; Forbes 1990, 1991). Mites of the genus *Arrenurus* attach to the larval form of damselflies in shallow water. It is at this point in its life that a damselfly accumulates much of the fat and muscle mass needed later in life for flight and male-male competition (Plaistow et al. 1999). It is possible that at this stage the mites are phoretic and do not affect the host (Rolff 2000); however, it has been reported that the parasite can decrease fat accumulation during the larval stage of the damselfly (Siva-Jothy 2000). When the damselfly transforms into its adult form, the water mites will develop a piercing mouthpart called a stylostome and begin to feed on the host (Abro et al. 1992). Larval mites are able to pierce the exoskeleton of the abdomen or thorax of a damselfly. This can interfere with water balance since haemolymph fluids may leak from the damselfly, although the pressure of this fluid in insects is usually low (Forbes et al. 1990). During the beginning of the season there are

fewer mites found on adult damselflies; however, as the breeding season progresses, the number increases until mites leave their odonate hosts to continue their life cycle (Forbes et al. 1990).

Fat content or body size can be an indicator of reproductive success, since body size affects survivorship, territoriality of males and fecundity of females (Forbes et al. 1990; Marden et al. 1989). Smaller damselflies may be more affected by parasitism (Joop et al. 2006) and have a greater change in body weight due to water mites than larger damselflies (Forbes et al. 1991). Also, the number of parasites has been shown to cause differential results in affecting damselflies. Heavily parasitized males (in relation to lightly parasitized males) respond less aggressively to other males, showing a decrease in territorial success. Heavily parasitized males also take fewer foraging trips, which could lead to a decrease in survivorship (Forbes et al. 1991). As a result, it is important to take into account the number of parasites, not just the absence or presence of the mites.

Studies relating to reproductive success of damselflies, due to degree of parasitism, have reported interesting results. There is no direct effect on mating since mites are not attached to the copulatory organs of the males or females (Forbes et al. 1990). The mating strategy of damselflies is for the males to scramble to compete for females when they are ready to copulate (Banks & Thompson 1985). In some cases non-mating males are more parasitized than copulating males (Forbes et al. 1991). This could be due to sexual selection by females or reduced ability of the males to assert normal reproductive behavior. Studies comparing the number of parasites and body weight have shown contradicting results. In *Enallagma erbiium* there is no correlation (Forbes et al.

1990); however, a study on *Coenagrion puella* shows that there is a negative correlation (Rolff et al. 2000).

The mating patterns and fitness of damselflies are well studied (Abro 1992; Fincke 1982; Forbes 1991, Siva-Jothy 2000; Waage 1979; Wade 1980). In *Enallagma hageni*, male-male competition instead of territoriality is an important factor in mating success. Smaller body size due to parasites could affect a male's ability to compete with other males for females (Fincke 1982). Males are also needed by the female in order to grab her after copulation so that she does not drown (Fincke 1983). This action of the male could also be affected by parasitism. Mating success of *Enallagma hageni* can be measured by the number of females a male can mate with or by how many viable offspring a male can produce with a female (Wade and Arnold 1980). Various male characteristics such as body weight, ability to forage for food, and success at clasping females affect its fitness against other males, and therefore, its ability to obtain mates in a random mating environment (Fincke 1982, 1984; Forbes 1990, 1991).

The present study investigates the effect of parasitism on *Enallagma hageni*. The number of water mites on copulating males versus single males was analyzed in order to find if mating success and body weight were reduced by parasitism. Reduced body weight of more heavily parasitized males would indicate a lower fitness in ability to forage. Parasitism could be a direct indicator of mating success as a trait that is selected against by females or it could be an indirect indicator of mating success due to a lower body weight affecting male-male competition, ability to guard mates while they oviposit and an ability to clasp females.

## Materials and Methods

Damselfly species *Enallagma hageni* on Sugar Island were caught using nets. On two separate days, seven days apart from each other, copulating pairs, as well as single males, were captured, stored in wax envelopes, and placed on ice in order to kill them. While frozen, the damselflies were observed with dissecting microscopes. The color, number and location of the water mites (whether they were on the abdomen or thorax), were recorded and written on wax envelope. Later, the mites were scraped off individual damselflies with a probe and the damselflies were weighed.

The sample size from day one was 170 damselflies including one 117 males and 63 females. On the second day, a sample size of 90 damselflies was obtained with 62 males and 28 females.

Results were entered into a spreadsheet in Microsoft Excel. Results were analyzed using t-tests and regression lines in SPSS 14.0.

## Results

### *Parasitism of Males and Females*

Of the total sample size of 260 individuals, 60 individuals had one or more mites attached to them. Prevalence of mites was calculated as how many individuals in a given sample size had one or more mites. A total of 26.8% of males had mites in the total male sample size of 179, and 14.8% of the total sample size of females of 91 had mites. Of the 78 mating males, 15 had parasites (prevalence of 19.2%) and of the 101 non-mating males, 33 had mites (prevalence of 32.7%). A chi-square test reveals that the difference in prevalence of mites on non-mating versus mating males is significant ( $\chi^2 = 4.03$ ,  $df = 1$ ,  $p < 0.05$ , refer to table 1). The percent of non-mating males with mites is significantly higher than the percent of mating males with mites.

Intensity of mites was calculated as the average number of mites per individual damselfly in a given sample. On average, males had 1.74 mites per individual and females had 0.98 mites per individual. This difference was not significant ( $t = 1.102$ ,  $df = 258$ ,  $p = 0.271$ ). The intensity of parasitism on males in copulation was 0.974 whereas the intensity on single males was 2.337 mites per damselfly. These results were not significant ( $t = -1.701$ ,  $df = 150.134$ ,  $p = 0.091$ ).

### *Mass of all Damselflies*

All of the individuals show a normal distribution of weight (Figure 1). The mean mass of all female damselflies is 0.02573 grams and the mean mass of all males is 0.01899. The difference between female body mass and male body mass is significant ( $t$

= -10.837,  $df = 116.248$ ,  $p = 0.000$ , Figure 2 and Figure 3), thus males and females have different body weights.

#### *Body Weight of Males with or without Parasites*

The relationship between mass and number of parasites shows a slight negative correlation (Figure 4). When considering the damselflies with at least one or more mites, the negative correlation is not significant ( $F = 0.450$ ,  $df = 1.46$ ,  $p = 0.506$ ).

The mean body weight of males with ten or more parasites is less than the mean body mass of males with less than ten parasites. Males with greater than ten mites have a mean body weight of 0.017 grams while the rest of the males have a mean body weight of 0.019 grams. These results are not significant ( $t = 1.695$ ,  $df = 158$ ,  $p = 0.092$ ).



## Discussion

The purpose of this study was to look at how *Arrenurus spp.* affected the mating success of *Enallagma hageni*. The prevalence and intensity of parasites on mating males and non-mating males was compared as well as the prevalence and intensity on female damselflies. The mass of the males was compared to the number of mites per male.

Male and female damselflies are found to be parasitized differentially. This could be because they are in different geographical areas when they develop. It could also be due to differences in immune responses by males and females. Females do not often come to the water unless to copulate so it could be that the difference in habitat could cause larval mites to fall off of females more readily than males. Further studies could focus on this aspect of differential parasitism in males versus females as this study did not have a large sample size of females. Studies on females would require capturing damselflies that were further from the water since this is where they are located.

Males in copulating pairs were less parasitized than non-mating males; however, the intensity of parasitism is not significantly different. This could reveal that males have a lower mating success when parasitized. Females may choose to mate with males even if the males have a few parasites; however, once males have more parasites this may be more unfavorable to the females. Male mating success could be affected directly by female choice (Waage 1979) since females may choose whether to mate with a male or not (Fincke 1983). The mites may be unattractive to the female; however, it is most likely that males that can fight parasites better will sire more offspring (Waage 1979) since they have a higher body weight and this implies an increased ability to conduct normal mating

behavior. Females choose males that will provide them with more offspring and also males that will sire more fit offspring. Parasitized males may not fit this profile due to some of the results of this study. Aside from female choice, males with a higher body weight can obtain more matings since they have an increased ability to clasp females and compete with other males for copulations.

As the number of mites on individual damselflies increased, the weight of the damselflies decreased slightly although not significantly. This reveals that mites could affect the body weight of damselflies and thus their fitness. Mites may affect flight patterns of damselflies if there are enough of them. Location of mites on the abdomen or thorax could affect the damselfly differently, so further studies on how mite location affects damselflies differentially could be valuable. The effect of number of mites on damselfly weight is stronger when considering males with more parasites, especially those with over 10 mites attached. Male damselflies may be able to combat the affects of parasitism when there are few in number and still be able to obtain as many mates and have better reproductive success through mate guarding. When there are many parasites on a damselfly, the males may need to spend more energy on combating parasitism than on male-male competition, foraging for food, or grabbing mates. A larger sample size of males with ten or more mites would probably reveal a stronger negative correlation. The present study collected test subjects later in the damselfly mating season, and as a result many mite could have already fallen off of the damselflies before they were collected. A study started earlier in the damselfly mating season and conducted on multiple days could reveal a stronger negative correlation and could provide data on how parasitism varies with time.

Mites may have evolved to increase their success on the *Enallagma* host by having more successful attachment mechanisms. Of the damselflies with parasites, it could be possible that the reason more damselflies have fewer mites is because the parasites have evolved a lower virulence by decreasing their prevalence on their host. When there are over one or two damselflies the success of the mites could become affected due to a strong effect on the host, thus they would evolve to have a lower prevalence. This co-evolution of host and parasite is a common occurrence in nature. It would be of interest to observe how the host and parasite interact and how the infection rate fluctuates from year to year. Both natural and sexual selection appear to work against parasitism in the present study.

## **Acknowledgments**

Thanks to my teachers Harvey Blankespoor and Steve Pruett-Jones for their agreement on this joint class project and for guidance in data analysis and project set-up. Thanks to Emily Kay for reviewing my paper and offering advice throughout the project and to David Gonthier for support and ideas. Thank you to the evolution class at the University of Michigan Biological Station for data collection and to UMBS for their resources and support. Many thanks to Akin for his statistical explanations and for help on the project.

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**Table 1**Chi-square test of mites on mating versus non-mating males of *Enallagma hageni*

	Mating Males	Single Males	Totals
Mites	15	33	48
No Mites	63	68	131
Totals	78	101	179

**Table 2**

Average number of mites on each damselfly group collected

	Average Number of Mites per Individual
Non-Mating Males	2.337
Mating Males	0.974
Females	0.98
Males	1.74

## Figure Legends

Figure 1. A plot of the normal distribution of all of the individuals of *Enallagma hageni* in the total sample of 260 individuals.

Figure 2. A histogram of the distribution of weight among female damselflies of *Enallagma hageni*.

Figure 3. A histogram of the distribution of weight among male damselflies of *Enallagma hageni*.

Figure 4. A regression comparing the weight of all males of *Enallagma hageni* to the number of mites on each individual.



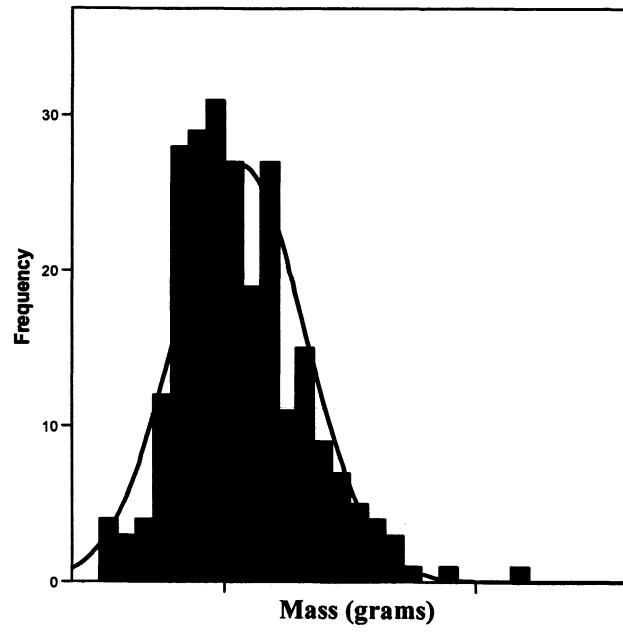


Figure 1

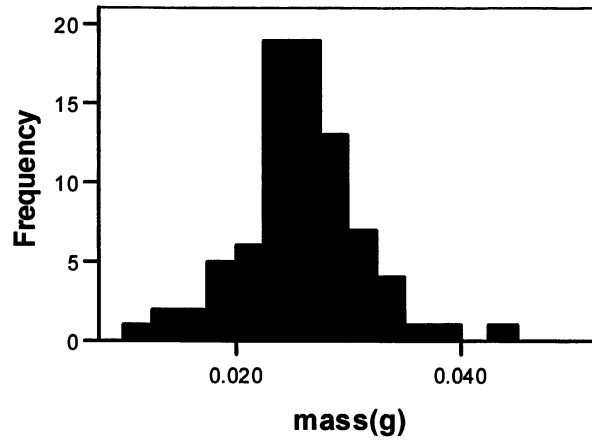


Figure 2

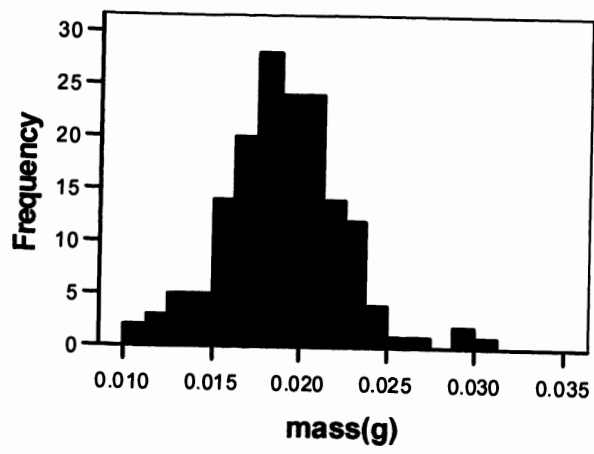
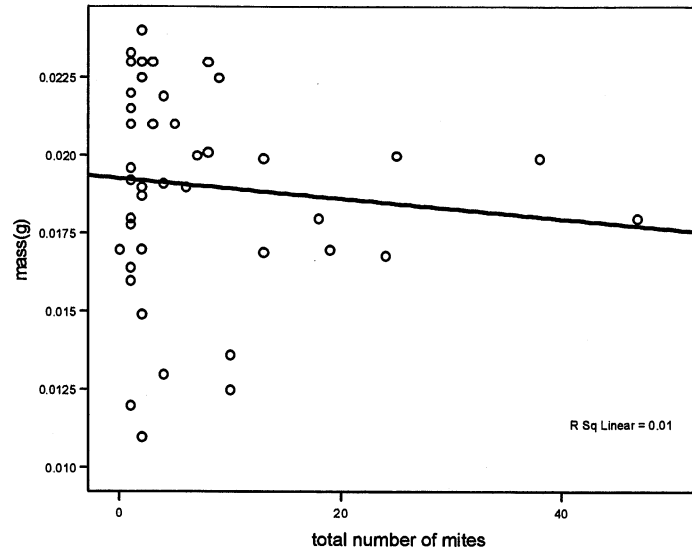


Figure 3



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## Results

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behavior. Females choose males that will provide them with more offspring and also males that will sire more fit offspring. Parasitized males may not fit this profile due to some of the results of this study. Aside from female choice, males with a higher body weight can obtain more matings since they have an increased ability to clasp females and compete with other males for copulations.

As the number of mites on individual damselflies increased, the weight of the damselflies decreased slightly although not significantly. This reveals that mites could affect the body weight of damselflies and thus their fitness. Mites may affect flight patterns of damselflies if there are enough of them. Location of mites on the abdomen or thorax could affect the damselfly differently, so further studies on how mite location affects damselflies differentially could be valuable. The effect of number of mites on damselfly weight is stronger when considering males with more parasites, especially those with over 10 mites attached. Male damselflies may be able to combat the affects of parasitism when there are few in number and still be able to obtain as many mates and have better reproductive success through mate guarding. When there are many parasites on a damselfly, the males may need to spend more energy on combating parasitism than on male-male competition, foraging for food, or grabbing mates. A larger sample size of males with ten or more mites would probably reveal a stronger negative correlation. The present study collected test subjects later in the damselfly mating season, and as a result many mite could have already fallen off of the damselflies before they were collected. A study started earlier in the damselfly mating season and conducted on multiple days could reveal a stronger negative correlation and could provide data on how parasitism varies with time.

Mites may have evolved to increase their success on the *Enallagma* host by having more successful attachment mechanisms. Of the damselflies with parasites, it could be possible that the reason more damselflies have fewer mites is because the parasites have evolved a lower virulence by decreasing their prevalence on their host. When there are over one or two damselflies the success of the mites could become affected due to a strong effect on the host, thus they would evolve to have a lower prevalence. This co-evolution of host and parasite is a common occurrence in nature. It would be of interest to observe how the host and parasite interact and how the infection rate fluctuates from year to year. Both natural and sexual selection appear to work against parasitism in the present study.

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**Table 1**

Chi-square test of mites on mating versus non-mating males of *Enallagma hageni*

	Mating Males	Single Males	Totals
Mites	15	33	48
No Mites	63	68	131
Totals	78	101	179

**Table 2**

Average number of mites on each damselfly group collected

	Average Number of Mites per Individual
Non-Mating Males	2.337
Mating Males	0.974
Females	0.98
Males	1.74

## Figure Legends

Figure 1. A plot of the normal distribution of all of the individuals of *Enallagma hageni* in the total sample of 260 individuals.

Figure 2. A histogram of the distribution of weight among female damselflies of *Enallagma hageni*.

Figure 3. A histogram of the distribution of weight among male damselflies of *Enallagma hageni*.

Figure 4. A regression comparing the weight of all males of *Enallagma hageni* to the number of mites on each individual.

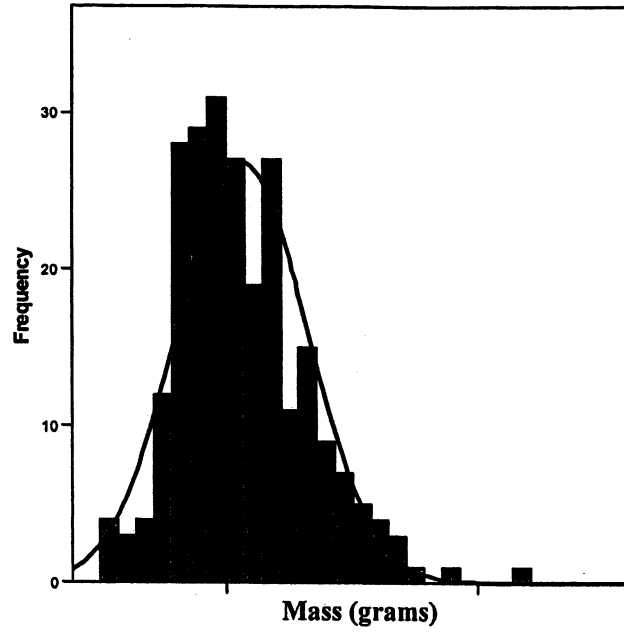


Figure 1

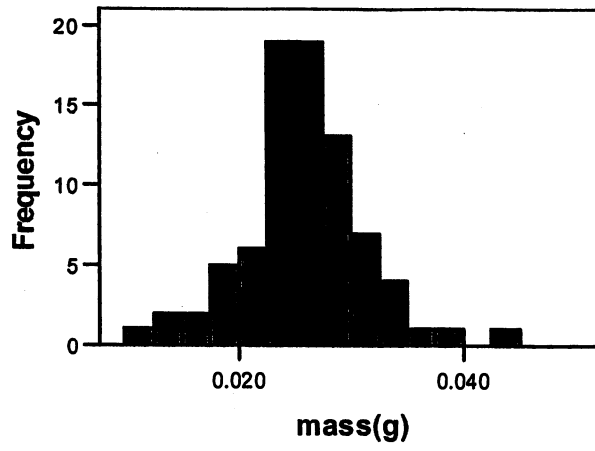


Figure 2

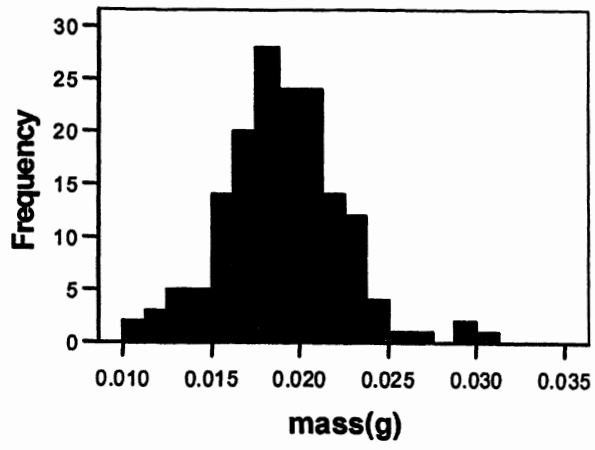


Figure 3

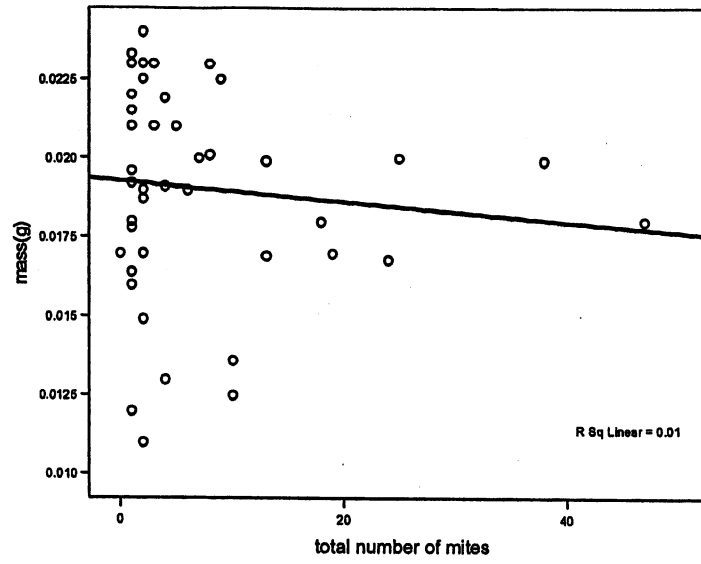


Figure 4