The Effect of Ectoparasites (*Arrenurus*) on the Mating Status of Damselflies (*Enallagma hageni*)

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Abstract

The Hamilton-Zuk hypothesis states that a female will chose a mate based on physical traits that indicate advantageous gene frequencies. In this study we investigated the effects of parasitism on mating success. Upon the emergence, parasitic water mites colonize on the abdomen of the adult damselfly *Enallagma hagenei.* We counted the intensities and prevalence of mites over the course of 2004 and 2007 from Sugar Island. In support of previous studies, a correlation between the number of water mites and mating success was found, but only in the 2004 sample. There is a relationship between weight and mating success, which indicates an alternative cue is used by female damselflies for mate choice. The male damselflies revealed a declining fitness over the course of the summer, where there were greater numbers of damselflies present, more mating and higher weights earlier in the mating season.

Introduction

Females seek indicators of good genes in males so to provide offspring with the better chances of survival. The Hamilton-Zuk Hypothesis states that females chose males based on secondary sex characteristics. The expression such phenotypes is limited by the parasites. The hypothesis reasons that a resistance to parasitism will be maintained due to co-evolution (Hamilton and Zuk 1982). To best ensure the survival of offspring, females should choose mates on a parasite level basis.

The relationship of co-evolution between parasite and host creates an ever changing frequency of genotypes. To better illustrate the matter, suppose both host and parasite populations have 2 alternate genotypes H, h and P, p. An individual host expresses the H genotype and has resistance to p but is susceptible to P. The opposite values apply to an h individual. Should parasite genotype p be most common, there is a selective advantage to the H genotype and the population expressing it increases. The system will hit an equilibrium in theory and reverse because there will be an advantage to be the P phenotype, this is an expression of a frequency-dependent selection.

Parasites live at the expense of their hosts and is a result of the co-evolutionary interaction between the two species (Siva-Jothy and Plaisow 1999). Parasites typically exhibit shorted generation cycles than their hosts, but water mites have at least the same life-cycle as its host (Forbes, Muma and Smith 2004).Mites evolve rapidly despite their life-cycle length. Under the coevolution, damselflies alter the time of emergence, which decreases the mean number of parasites(Stechmann in Rolff 2001). In response, the mites are capable of evolving fast enough to shape its hatching pattern to match the emergence of its host (Forbes, Muma and Smith 2004). The parasitic stage of the water mite evolved due to the genetic advantage. Sister species of Arrenurus who lost the parasitic stage displayed a significantly lower heterozygosity, indicating that the host-mediated dispersal is important for maintaining genetic diversity (Rolff 2001). In addition to altering life histories, damselflies partake in behavioral defenses. Parasitized damselflies groomed and tended more than damselflies without mites (Leonard, Forbes and Baker 1999). Females can also dislodge mites by wetting them during oviposition (Forbes, Muma and Smith 2004).

Life history of the water mite

Water mites inhabit most freshwater habitats and of those parasitic mites, almost all use insects as hosts while in the larval stage (Smith 1988). The species *Arrenurus* parasitizes adult dragonflies and damselflies (Mitchell in Forbes 1991). Shortly after hatching, mites must locate a

host. Mites swim to the local vegetation where damselflies perch (Leonard et al 1999). The mites climb on to the damselfly and attach by piercing the exoskeleton with their chelicerae, where it secretes a substance that cements to the host a stylostome forms within 10 min of attaching(feeding tube). An *Arrenurus* mite typically lives on host 10 days before detaching (Smith 1988). During the 10 days, the mites engorge, swelling 500 times its original size (Rolff 2001). Once the mite has grown sufficiently, they drop off to complete their life cycle only in specific waters as free-swimming nymphs and then adults (Robb and Forbes 2006).

Life history of the damselfly

Damselfly live among vegetation of freshwater habitats (Smith 1988). After hatching from eggs, damselflies enter a nymph stage where they leave the water area. Once sexually mature, the damselflies return to the original site of hatching to mate. *E. hageni* participate in "scramble" competition for males(Fincke 1986). The ponds are typically heavily skewed towards males promoting the heavy local competition. The males defend a territory among the vegetation that act as oviposition sites for the females. During copulation, males clasp the female behind the head and the female brings her ovipositor up to the male's genitals, which are located on the just below the thorax. After copulation, the male guards the female as she deposits the newly fertilized eggs on vegetation growing out of the water (Rollf et al 2001).

Objective

The purpose of this study is to investigate the effect of mites on the mating success and fitness of *Enallagma hagenei.* The combination of weight and mating success will be the measures of fitness. We plan to assess the effect of time to mite prevalence and mating status as well. Fresh water habitats containing damselflies are greatly available in this area, easing the course of this study. The evolution of the interaction between the two organisms can easily be studied through the distributions of mites and apparent fitness of the damselflies. The Hamilton-Zuk hypothesis predicts that females will not chose males that are heavily parasitized. We hypothesized mite load not to be random but a clumped distribution. We predicted an effect of mites on the mating status of males where males with high levels of mites will mate infrequently. We also hypothesized an effect of time and fitness where damselflies are predicted to weigh less later in the summer, as well as mate less frequently. Good fitness also includes longevity. We predicted fewer damselflies to be caught later in the summer due to decreased population sizes over time.

Materials and methods

The damselflies were collected from Sugar Island, Michigan on four occasions between the years 2004 and 2007. Capture was orchestrated with butterfly nets and the caught damselflies were stored in glassing envelopes. We killed the damselflies by leaving them in the freezer for at least one hour. Day 1, July 9, 2004 of the 104 damselflies collected, 28 were mating. Day 2, July 16, 2004, 299 damselflies were collected and 22 were mating. On day 3, July 23 2004, 25 of the 124 were mating. Day 4, July 24, 2007 yielded 19 mating pairs in a catch of 138 damselflies. After killing the damselflies, we counted the number of parasites with dissecting microscopes as well as determine the sex. All the mites were removed from the damselflies and the weights of the damselflies were recorded.

Data was originally entered in Microsoft excel and calculations were completed with SPSS 14.0. We calculated the intensity (average number of mites per damselfly) and prevalence (percent of parasitized damselflies) of the mites on the damselflies. A Poisson distribution was calculated using the intensities of parasitism, which serves as the expected values of parasitism the population would experience. By running a chi-square, we computed whether the observed

number of parasitized damselflies differed from the calculated one. Comparisons of data were primarily conducted with nonparametric tests because the data failed the test of normality. The independent samples were computed with Mann-Whitney *U* tests and the paired samples were computed with Wilcoxon paired sign tests.

Results

We observed 35.29 % of male damselflies in 2004 from Sugar Island were parasitized and that 16.56% of males in 2007 were parasitized. Of the mating males in 2004, 33.3% had mites. Of the non-mating males, 35.6% had mites. Non-mating males reported greater numbers of mites than mating males in the 2004 sample ($Z = -4.401$, $p < 0.001$). Of the mating males in 2007, 5.30% had mites, this was 1 of 19 mating pairs caught. Of the non-mating males, 18.10% had mites of 138 individuals caught. Non-mating males reported no difference in mite numbers than the mating males in the 2007 sample ($Z = -1.000$, $p = 0.317$). The prevalence of mites decreased with duration of the summer as is illustrated with these comparisons.(Table 1) July 9 reported more mites than July 16 ($Z = -3.776$, $p < 0.001$). July 9 counted higher numbers of mites than July 23 ($Z = -3.465$, $p = 0.001$). July 9 also had more mites than July 24 ($Z = -6.343$, $p < 0.001$). July 16 had greater numbers of mites than July 24 ($Z = -3.695$, $p < 0.001$). July 23 reported higher numbers of mites than July 24 ($Z = -3.051$, $p = 0.002$).

There is evidence that fitness decreases with time (Forbes, Muma and Smith 2004). What is the effect of time on the sugar island population? Is there a correlation between weight and mating success? We found that larger males mated more than smaller males ($Z = -3.180$, $p = 0.001$).

Weights were only taken for part of the sample collected on July 9 and July 16. Comparing these two samples, there seems to be an effect of time. The July 9 sample weighed more than the July 16 sample ($Z = -4.679$, $p < 0.001$). There is a marginally negative correlation between weight and number of mites for mating males $Y = -5 \times 10^{-5} X + 0.021$ (figure 2). There was a marginally negative correlation between weight and number of mites for non-mating males as well $Y = -1.1 \times 10^{-8} X + 0.019$ (figure 3).

Discussion

We observed an effect of time, as hypothesized, verifying the decreasing weights, caught mating pairs and sheer numbers of the damselflies over the course of the summer. These findings are in accordance to Forbes, Muma and Smith (2004). The diminishing prevalence and intensity of mites can be accounted for seasonal changes in the case of 2004, but comparing July 23, 2004 and July 24, 2007 suggests and effect of year as well. Damselflies were collected at the same point in the season, which reveals the effect of time. Merely reporting the date must not explanation enough of the variation, the time of day and condition of weather could clarify the differences. Lower survival of heavily parasitized males may account for the lower mite numbers later in the season, leaving primarily the mildly parasitized males (Forbes, Muma and Smith 2004). It may also be the decrease in fitness of later emerging mites; greater resistance from the hosts and fewer available hosts decreases the prevalence of mites (Forbes, Muma and Smith 2004). In fact, the number of mites is related to the time of emergence and nutritional condition (Forbes and Baker 1990).

In the entirety of the 2004 data, mite number influenced the probability of a male copulating. This observation is the most historically prevalent result and is congruent with studied by Forbes (1991). Recent research suggests that mite detachment was not considered in the parasite prevalence in these studies. Counting parasites a few days after emergence, before mites detached produced no effect on mating success (Rolff, Antvogle and Schrimpf 2000).

Analysis of birds and amphibians further supports the claim that parasitism does not affect male mating success (Hamilton and Poulin 1997 in Rolff, Antvogle and Schrimpf 2000). Colleting multiple times long after the mites initially attached themselves may have given us inaccurate data. Unfortunately, sample sizes were far too small during the 2007 collection to infer conclusions regarding mite count and mating status.

Measuring the exact prevalence of mites is ideal. Our study, like many others, did not and can not account for detached mites. To better estimate mites numbers, future studies should count them soon after damselfly emergence. There is tissue destruction where the stylostome forms (Smith 1988). In addition to counting mites earlier in the season, study of the damage on the thorax should be completed, which would act as insurance for potential mite detachment. We detected an effect of weight and mating success. Larger males mated more frequently than smaller males. Parasite load has been negatively correlated with lipid content (Rolff 1990). Our data supports this conclusion. Mites both weigh down and consume damselflies, which required greater energy. Lipid content is correlated with intraspecific competition success for territory (Forbes, Muma and Smith 2004). With no territory, males have little chance of obtaining a mate (Rolff and Siva-Jothy). A difference in mass cannot totally account for mite load differences (Forbes and Baker 1990). Daily activity, especially the war of attrition competitions between males will decrease lipid contents of male damselflies. We indirectly determined the lipid content through weight. A true measure of fat would better describe the true relationship between parasitism and weight. Not all of the masses were recorded for the damselflies in 2004 and none in 2007, an additional flaw in the methodology of this study. Conclusions based on weights were computed with sample sizes considerably smaller than the rest of the samples used in this study. If parasitism does not affect female choice as Rolff et al (2000) suggests, some other behavioral or biological cue must influence mate choice. Territory as a result of size may account for some of that variance. It seems that heavily parasitized males are capable of mating with females, so males must opt for mating tactics which are energetically less expensive but have lower mating returns(Forbes, Muma and Smith 2004).

Summary

Mite intensity seems to influence mating status. Females must also mediate sexual selection according to other visual cues such as weight, which also had a significant relationship with mating success. Our conclusions refute recent convictions and support the Hamilton-Zuk hypothesis; this may be due to flawed methods and small sample sizes.

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Descriptive Statistics

	N	N Parasitized	Prevalence	Intensity
July 9, 2004	104	53	50.96%	4.5962
July 16, 2004	299	84	28.09%	2.4849
July 23, 2004	124	39	31.45%	1.8952
Combined 2004	527	186	35.29%	2.7628
July 24, 2004	157	26	16.56%	.6497

For each of the following dates N number of male damselflies were caught and N had parasites. Prevalence is the percent of damselflies that were parasitized. Intensity is the mean number of parasites each male damselfly had.

Combined 2004

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	.00	341	64.6	64.7	64.7
	1.00	64	12.1	12.1	76.9
	2.00	23	4.4	4.4	81.2
	3.00	15	2.8	2.8	84.1
	4.00	10	1.9	1.9	86.0
	5.00	6	1.1	1.1	87.1
	6.00	8	1.5	1.5	88.6
	7.00	5	.9	.9	89.6
	8.00	1	.2	.2	89.8
	9.00	2	.4	.4	90.1
	10.00	3	.6	.6	90.7
	11.00	2	.4	.4	91.1
	12.00	2	.4	.4	91.5
	13.00	1	.2	.2	91.7
	14.00	1	.2	.2	91.8
	15.00	6	1.1	1.1	93.0
	16.00	3	.6	.6	93.5
	17.00	2	.4	.4	93.9
	18.00	1	.2	.2	94.1
	19.00	3	.6	.6	94.7
	20.00	6	1.1	1.1	95.8
	21.00	4	.8	.8	96.6
	22.00	4	.8	.8	97.3
	23.00	3	.6	.6	97.9
	24.00	1	.2	.2	98.1
	25.00	2	.4	.4	98.5
	26.00	1	.2	.2	98.7
	27.00	1	.2	.2	98.9
	33.00	1	.2	.2	99.1
	36.00	1	.2	.2	99.2
	44.00	1	.2	.2	99.4
	53.00	1	.2	.2	99.6
	56.00	1	.2	.2	99.8
	62.00	1	.2	.2	100.0
	Total	527	99.8	100.0	
Missing	System	1	.2		
Total		528	100.0		

This is the distribution of mites. 341 or 64.7% of the male damselflies had 0 mites.

Parasite Frequency	Expected	Observed	Chi-Square
0	33.26488735	341	2846.872696
1	91.90090429	64	8.470650712
2	126.9473141	23	85.11439719
3	116.9057816	15	88.83040839
4	80.7439007	10	61.98238434
5	44.6142349	6	33.42115224
6	20.54262446	8	7.658097855
≥ 7	12.08133189	60	190.0617229
Total	527	527	3322.41151

The Poisson distribution predicts the number of random events will produce 0, 1…mites on a population of damselflies. A Chi-square test used the distribution and concluded that the distribution of mites is not random but clumped.

Figure Legends

1. Intensity of mites per date
2. Overall Non-mating weight vs mite count
3. Overall mating weight vs mite count

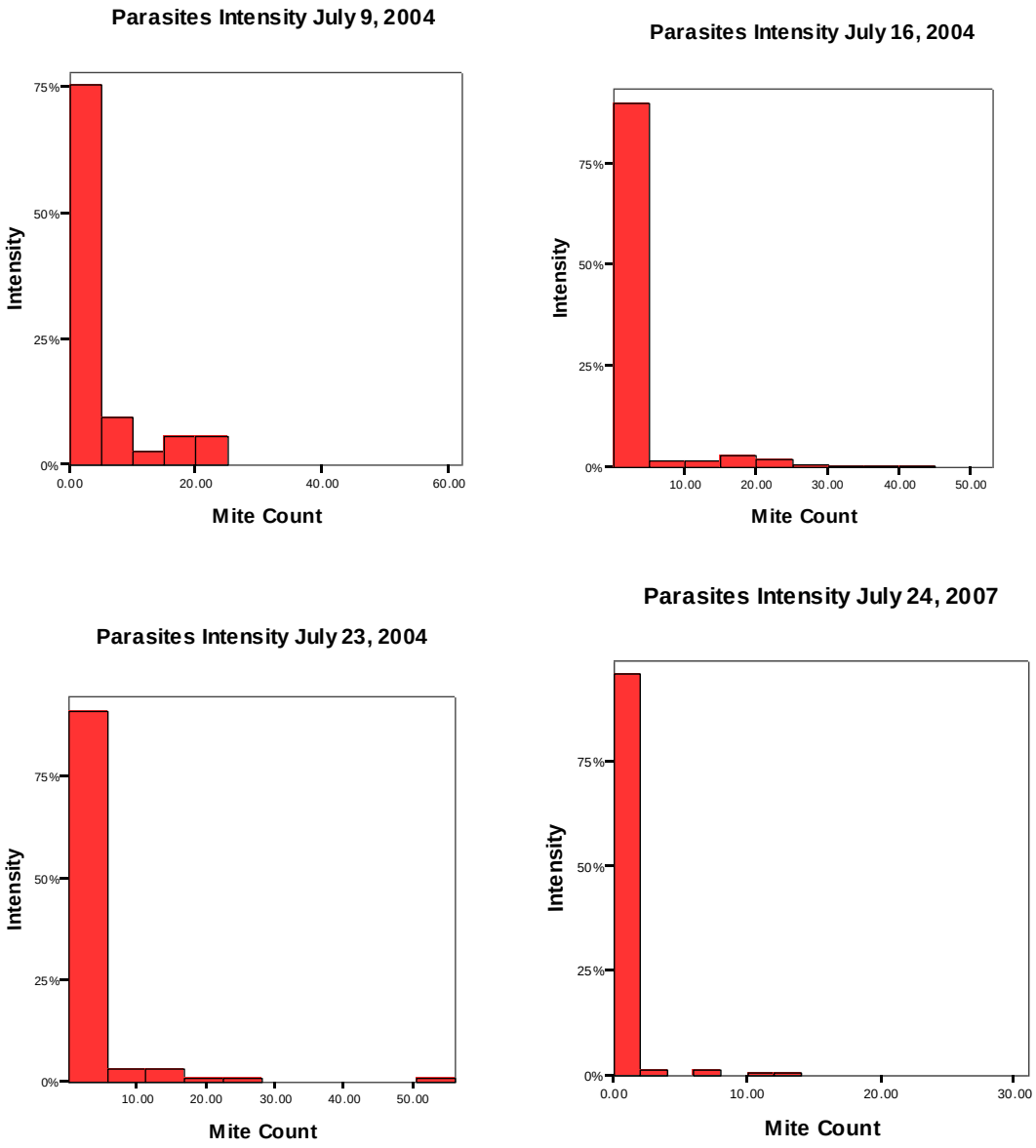
Parasites Intensity July 9, 2004
Intensity
75%
50%
25%
0%
0.00
20.00
40.00
60.00
Mite Count
Parasites Intensity July 16, 2004
Intensity
75%
50%
25%
0%
10.00
20.00
30.00
40.00
50.00
Mite Count
Parasites Intensity July 23, 2004
Intensity
75%
50%
25%
0%
10.00
20.00
30.00
40.00
50.00
Mite Count
Parasites Intensity July 24, 2007
Intensity
75%
50%
25%
0%
0.00
10.00
20.00
30.00
Mite Count

Figure 1

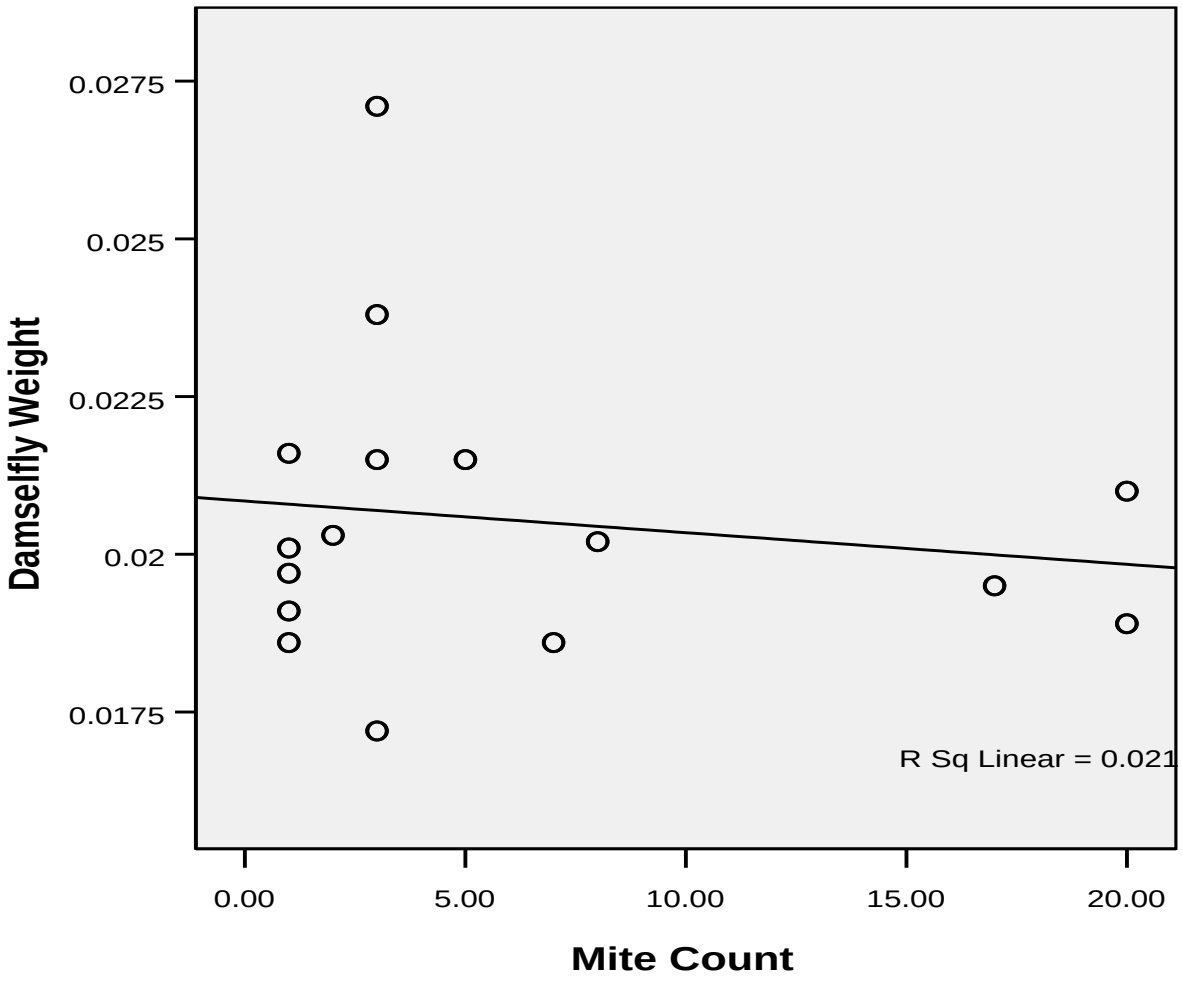
Damselfly Weight
0.0275
0.025
0.0225
0.02
0.0175
R Sq Linear = 0.021
0.00
5.00
10.00
15.00
20.00
Mite Count

Figure 2

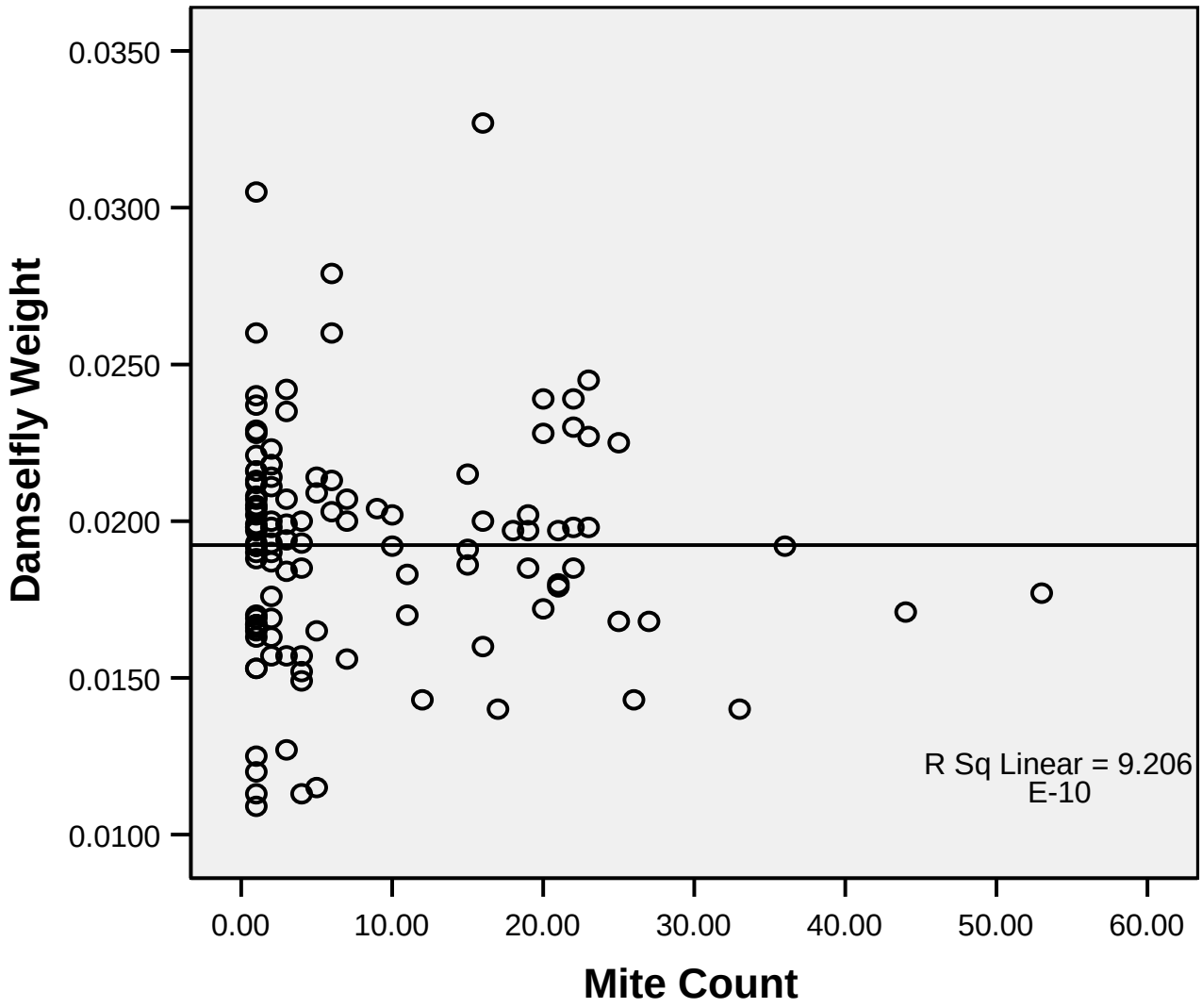
0.0350
0.0300
0.0250
0.0200
0.0150
0.0100
Damselfly Weight
R Sq Linear = 9.206
E-10
0.00
10.00
20.00
30.00
40.00
50.00
60.00
Mite Count

Figure 3