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Effects Of Food Deprivation, Body Size, And Egg Load On The Mating Behavior Of Female Japanese Beetles, *Popillia Japonica* Newman (Coleoptera: Scarabaeidae)

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Effects of food deprivation, body size, and egg load on the mating behavior of

female Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae)

(TITLE)

BY

Natasha Tigreros

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Master's Thesis

**Effects of food deprivation, body size, and egg load on the mating
behavior of female Japanese beetles, *Popillia japonica* Newman
(Coleoptera: Scarabaeidae)**

Natasha Tigreros

Eastern Illinois University

ABSTRACT

Female condition may play an important role in the mating frequency of species that mate at foraging areas. For example, in species where feeding females are more easily encountered or less resistant to mating than females that are performing other activities, low energy levels could increase mating activity. We studied the relationship between female condition and mating activity in the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae) in a laboratory study. We altered condition by depriving some females of food for one day and comparing their mating behavior to well-fed females. Our results showed that the effect of condition on mating behavior only became apparent when we considered female body size and fecundity status: well-fed, relatively large females, well-fed, relatively fecund females, and unfed, relatively small females mated more frequently than their counterparts. These patterns may be a result of food deprivation altering the costs and benefits of mating for females of different body sizes and egg loads, and perhaps reflect a need for females that are about to oviposit needing to maximize their food intake.

DEDICATION

Dedico esta tesis a mi madre y a Perita que siempre me han parado tantas bolas y animado con mis inquietudes científicas.

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CHAPTER I

Polyandry in insects

Male and female mating strategies and interests differ and may be in conflict because of their initial, asymmetric parental investment (anisogamy): a male's gametes are small and many, while a female's gametes are big and limited. Consequently, multiple matings can increase male fitness and males may be selected to mate with multiple females. Females, however, often store sperm and can fertilize many of their eggs from one only copulation, and thus, their fitness is less likely to benefit from mating repeatedly (Parker 1979). Nevertheless, females of most insects actually mate multiple times (Arnqvist & Nilsson 2000; Härdling & Kaitala 2005) and in many groups of insects either direct or indirect benefits have been shown to exist for polyandrous females. Female's lifespan is enhanced when material benefits are involved in matings (nuptial feeding and nutritional resources passed with sperm) (see review by Vahed 1998); also, females can reduce predation risk and sexual harassment when mating, or increase the genetic diversity of offspring mating with different males (see reviews in Walker 1980; Thornhill & Alcock 1983). Finally, an important benefit of mating multiple times may come with the sperm replenishment because sperm may lose viability over time, or male may fail to transfer viable sperm with a mating (Simmons 2001); in these circumstances, repeated mating can increase a female's fecundity and/or fertility (Ridley 1988).

However, mating has its costs, and how often a female mates has been suggested to reflect a trade-off between the costs and benefits of mating (Arnqvist & Nilsson 2000). These costs vary from time and energy devoted to matings to the increase in the risk of injury, parasites and diseases (see Daly 1978; Simmons 2001; Arnqvist & Rowe 2005). Female mating frequency may increase as a result of the high costs of resisting male mating attempts (Arnqvist & Nilsson 2000). This "convenience polyandry" mating system (Thornhill & Alcock 1983) has been well studied in water striders (Rowe 1992); essentially, the female reluctance to mate decreases when it becomes more expensive to repel the mating attempts than to accept the extra copulations.

A female's condition and experience may affect the costs and benefits of a mating at a particular point in her life. Female fecundity and mating and oviposition history are important factors that may cause a female's receptivity to change. In some species, more fecund females are more willing to mate (for example Woodhead 1981; Wedell & Cook 1999). In others, the receptivity to additional copulations has been related to a female's oviposition history; females that have laid more eggs have less stored sperm and thus, are more likely to re-mate (Eady et al. 2004).

Mating frequency in insects seems to be partially controlled by female behavior via allowing or rejecting male mating attempts. In insects, females' rejecting behavior varies from struggling, running, flying away, dropping off the plant, and pushing down their abdomen (See Woodhead 1981; Row 1992; Ringo 1996), to simply avoiding areas where harassment is common (Stone 1995; Clutton-Brock & Parker 1995). For example, because male harassment may interfere with female's foraging activity in the solitary bee (*Anthophora plumipes*), females may forage from the flowers inside the plant's growth, even though these flowers are not as profitable as the outer flowers, because inside flowers are less frequented by males (Stone 1995).

Japanese Beetles Biology

Upon emerging, virgin female *P. japonica* release a sex pheromone that is strongly attractive to males. It was first noticed by Smith and Hadley (1926) how males congregated in to "balls" around an emerged female. However, females stop using this pheromone after their first mating (Ladd, 1970a) and the non virgin females continue to mate on the food plants, on which the aggregation of adults is probably caused by plant kairomones (Loughrin *et al.*, 1995).

Japanese beetles are a promiscuous species with both male and females capable of mating repeatedly during their lives. Pairing patterns seem to be at least partially related to size; with a large male and female advantage in matings. In a field study, Switzer et al. (2001) found that paired females were larger than unpaired females and paired males were larger

at 0700 and smaller at 1000. Males tend to mate with a larger female when presented with a small and a large female option (Saeki, et al., 2005a).

Like in many animal species (see Bagemihl 1999) male Japanese beetles have homosexual pairing both in the laboratory and in the field. In the field, from 3-6% of the matings are male-male couples (Switzer et al., 2004). The formation of homosexual pairs is related to body width (Switzer et al., 2004; Switzer et al., unpublished): the male on the top tends to be smaller than the male on the bottom (Switzer et al., 2004). Furthermore, although males of all sizes are likely to mount other males and extend their aedeagus, mounts are longer and the aedeagus extension more likely to occur if the mounted beetle is larger than the mounting beetle (Switzer et al., unpublished). This trend of males mating with larger males is probably the result of males using size as a cue to distinguish females; although females tend to be larger than males, there is an overlap between male and female sizes (Switzer et al, 2004; Switzer et al, unpublished).

After a brief copulation of about 2 minutes, Japanese beetles have long periods of postcopulatory association; males can remain on top of a female from a few minutes to several hours (Saeki et al, 2005b). This postcopulatory association could be interpreted as a "mate guarding" behavior because they have approximately a 70% last male sperm precedence (Ladd 1970b), the sex ratio is male-biased, and the paired male does defend the female from other males while paired. In the laboratory, mate guarding durations were longer with bigger females and at higher densities (Saeki et al, 2005b).

Some evidence for female resistance of male mating attempts exists. Although most of the time copulation occurs shortly time after a male mount a female, females can resist successfully the male aedeagus insertion for several minutes (Switzer et al, unpublished). Also, on the food plants, females have been observed to crawl, fly away and drop off when males approach.

Japanese beetle behavior may change depending on temperature or time of day (Table 1).

In the field, heterosexual pairings are more common in the morning and the evening (Switzer et al, 2001), but homosexual pairings are more likely to occur in the late afternoon (Switzer et al, 2004). Both heterosexual and homosexual pairings are more common at lower temperatures (Switzer et al 2001, 2004). In the lab, mate guarding duration is affected by temperature, being shorter at higher ambient temperature than at lower temperature (Saeki et al, 2005c). In addition, although male-male contest frequency is not affected by temperature, contests tend to be more frequent during the middle of the day (Kruse and Switzer 2007).

Table 1. Japanese beetles behavioral patterns associated with time of day.

Time of day	Heterosexual mounts ¹	Homosexual mounts ²	Fights ³	Sex ratio ¹
Morning	+	-	-	=
Noon-Afternoon	-	+	+	=
Evening	+	-	-	=

Sources: 1. Switzer et al. 2001; 2. Switzer et al. 2004; 3. Kruse and Switzer, submitted.

The variation in the frequency of sexual and fighting behavior throughout the day (Table 1) may be explained by changes in male behavior, female behavior, or both. First of all, the change of the mating frequency of males and females can be an effect of males remaining with females longer following copulation in the morning and evening. As stated previously, temperature is negatively correlated with mount duration and lower temperatures usually occur during the morning and evenings. Thus, if males stay in amplexus for shorter during the afternoon, one might expect that their mating attempts would be more frequent, leading to higher rates of male-male (homosexual pairing) and more fights between single and paired males.

Secondly, the availability of receptive females may be an important factor affecting the mating patterns (Kruse & Switzer 2007). The increase of homosexual mounts and male-male fights at the time of the day where heterosexual mounts are less frequent, without a significant change in the sex ratio (Switzer et al. 2001), suggests that although females are present, they are not as available to mate (in contrast to males that attempt to mate throughout the day). If females do vary in receptivity, the variation could be explained

by their fecundity status and/or the cost of mating at a particular time of the day (costs related with temperature, foraging status, harassment rate, etc).

On the food plants, a female decision to mate may be influenced not only by how fecund she is but also by her feeding status. Japanese beetle females eat while they mate on the food plants (Fleming 1972; Kreuger & Potter 2001); thus, one may predict that hungry females will be more likely to stay on the food plant and accept a mating even if they have lower egg loads. On the other hand, a female without or with fewer eggs that is well fed will not lose too much energy if she has to stop eating; therefore, one might predict a nongravid, well-fed female will be more likely to reject mating attempts. For gravid females, feeding status is predicted to not affect the mating acceptance because they are ready to mate. In short, the feeding condition of a female and her egg load may change her decision to mate.

Although female behavior may at least be partially responsible for some of the observed daily patterns (Table 1), studies so far have focused only on male mating behavior without consideration of the female role. Thus, in my thesis I am going to examine how female receptivity to mate varies with her fecundity status and her feeding history.

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CHAPTER II

Effects of food deprivation, body size, and egg load on the mating behavior of female Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae)

INTRODUCTION

Female reproduction is limited mainly by her ability to obtain resources to produce offspring (Borgia 1979). This need to obtain resources drives an interesting dynamic between a female's energy levels, egg production, and her availability or receptivity to mating. For instance, because mating and foraging are mutually exclusive in many insects (e.g. Krupa & Sih, 1993; Odendaal et al., 1989; Stone, 1995), females often face a trade-off between time and energy devoted to collecting food and mating. As a consequence, one may expect females with lower energy levels to mate less frequently in order to increase the time available to obtain and ingest food. For example, Rowe (1992) demonstrated that hungry female *Gerris buenoi* mated half as often as recently fed females, and thus increased their foraging time. Alternatively, if mating does not interfere with female feeding activity, low energy levels could have a positive effect on mating activity, especially in those species in which feeding females are more easily encountered or less resistant to mating (e.g. Rowe et al., 1996; Watson, 1993).

A female's egg load may also affect her mating behavior. For instance, females with more eggs may mate more frequently, either because males prefer to mate with more fecund females (e.g. Monaghan et al., 2001; Sargent, et al., 1986) or because more fecund

females are more willing to mate (Woodhead 1981; Wedell & Cook 1999). In contrast, females with fewer eggs could be expected to invest more time eating than those with higher egg loads in order to obtain the resources necessary to produce more eggs. Therefore, for species in which female food intake affects mating activity, the egg load of a female may affect mating behavior both in a direct manner (females with more eggs are preferred or more receptive to mate) and in an indirect manner (females with fewer eggs will spend more time eating and that will either increase or decrease their mating activity).

In this study, we investigated how a female's state (defined broadly as female size, energy level, and egg load) affected mating patterns in Japanese beetles (*Popillia japonica*). As adults, Japanese beetles eat over 300 species of plants (Fleming 1972). Japanese beetles have a promiscuous mating system with both male and females capable of mating repeatedly during their lives (Fleming 1972; Tigreros & Switzer, unpublished data) and in which females periodically return to the soil to lay eggs (Fleming 1972). Upon emergence, virgin, adult females emit a sex pheromone to attract males (Ladd, 1970). Nonvirgin females mate on the food plants, on which adults aggregate as a result of plant kairomones (Loughrin *et al.*, 1995). Japanese beetle females eat while they mate on the food plants (Fleming 1972; Kreuger & Potter 2001; Tigreros & Switzer unpublished data), and in the field, most of the females (90%) have been observed to re-pair within one hour of separating from their original pair (Tigreros & Switzer unpublished data). Matings consist of a brief copulation of about 2 minutes, and a postcopulatory association that can last from a few minutes to several hours (Saeki *et al.*,

2005a). The size of the female appears to affect mating; paired females in the field are bigger than single females (Switzer et al., 2001) and males choose bigger females when given an option (Saeki et al., 2005b).

Mating behavior of Japanese beetles varies throughout the day (Switzer et al., 2001). In the middle of the day, there is less heterosexual pairing (Switzer et al. 2001), but more homosexual pairing (Switzer et al. 2004) and more male-male contests for females (Kruse and Switzer 2007). Furthermore, these heterosexual mating patterns seem to be unrelated to sex ratio (Switzer et al. 2001). Although costs of pairing in males may be responsible for at least some of these patterns (Saeki et al. 2005c), another possibility is that although females are present, perhaps they are not as available to mate or as receptive to mating during the afternoon.

Many intrinsic and extrinsic factors could be affecting female availability on the food plants. This study examined whether female condition affects mating activity. Because female Japanese beetles do not face a dramatic trade-off between mating and eating, we predicted that females with relatively low energy levels would spend more time eating and not moving, and therefore would be more available to mate than well-fed females. Also, we expected that females with fewer eggs would eat more frequently and as a consequence would mate more than females with more eggs.

METHODS

One day prior to their use in the trial, we collected beetles from a variety of food plants from horticultural areas in Coles County, Illinois, USA. To control for their recent mating history, we used only those beetles that were paired at the time of collection. In the lab, we separated males from females on the basis of foreleg morphology (Smith and Hadley 1926) and measured the size of females. Size was determined by measuring the maximum body width (which occurs just posterior to the pronotum) with a dissection scope with an ocular micrometer. This measure of body size correlates well with other morphological measurements and thus is a good measure of overall body size (Van Timmeren et al. 2000).

To test for the effect of food deprivation, test females were randomly assigned to two experimental groups. In the "Well-fed" group, females were given leaves from littleleaf linden (*Tilia cordata*, a preferred food plant for *P. japonica*; Miller & Ware, 1999) *ad libitum* as well as paper towels saturated with water. In the "Unfed" group, females were provided only with paper towels saturated with water. Experimental males were held in conditions similar to the Well-fed female treatment. These pre-trial treatments lasted for approximately 20 hr; pilot studies indicated that this time period does not lead to significant differences among female egg loads (well fed females: median = 2, range = 0-2, N = 27; unfed females: median = 2, range = 0-11, N = 27; Mann-Whitney U = 322, z = 0.75, P = 0.46, N = 27).

For trials, we wanted to recreate an environment similar to natural conditions (i.e. small aggregations of beetles on the food plants), but we also needed to observe the behavior of individual beetles. Therefore, we placed groups of eight beetles (four males/four females) in small acrylic boxes (16 x 16 x 16 cm), with small branches of linden leaves and a vial of water with a cotton wick. To provide similar mating conditions among acrylic boxes, all the food plants had the same number of similar-sized leaves. In addition, we wanted to provide a range of female sizes and yet also wanted to keep the size range and average female size similar among trials. Consequently, we did not use those relatively rare females of very large or very small size in our experiment. To identify an individual, we glued a colored queen bee tag (Thorne, Ltd.) to its pronotum. Because mating behavior may be affected by environmental conditions (Switzer et al. 2001, Switzer et al. unpubl. data), we ran the experiments in an environmental chamber at constant temperature (25°C), light level (1850 fc), and humidity (60% RH). Females were placed into the boxes 15 minutes before the males were added so females had time to disperse and begin eating. We then introduced the males and started the observations. At 20-min intervals, we recorded the mating status (paired or single) and activity (crawling, eating), for each individual. The observations for each trial lasted 5 hours.

In order to determine the treatment effect on the mating behavior, we used each acrylic box as a replicate in the statistical analysis. We measured crawling activity as the total number of observation periods that females of the same trial were crawling, divided by the total number of observation periods and averaged over the females in the trial; we calculated this number without regard to pairing status and then again for females when

they were paired and when they were single. We followed the same procedure to calculate eating activity. Following Rowe (1992), we defined pair duration as the mean duration of pairs of each replicate; this number therefore included both copulation time and post-copulatory guarding time. To avoid pseudoreplication, we only included the first pair for both females and males. Mating frequency was determined as the total number of matings that occurred in each box divided by four. Total mating activity was defined as the proportion of time females were paired and was calculated as the sum of the number of mating pairs on each of the observation periods divided by the potential number of pairs (i.e. the total number of females x the number of observation periods).

To examine the effect of egg load on female mating behavior, we dissected females after the trial and counted the number of mature oocytes. Because analysis showed there was no block effect in our data, we pooled the replicates for each treatment together and correlated individual female behavior (# of matings, duration of the first mating, and proportion of time females were mated) with her body size and egg load for each of the two treatments.

Statistics were calculated using SPSS statistical software Version 13.0 (SPSS, Chicago, IL). Parametric tests were used unless distributional tests could not be met even following transformation. Means are given as $\pm$ SE.

RESULTS

Food deprivation effect

Food deprivation had an effect on female foraging behavior: unfed females fed proportionally more often than fed females (well-fed females = 0.23 ± 0.03 ; unfed females = 0.36 ± 0.03 ; $t = 2.91$, $df = 28$ $P = 0.007$). Also, the overall crawling activity was less for unfed females than for well-fed females (well-fed = 0.50 ± 0.04 ; unfed females: mean = 0.35 ± 0.03 ; $t = 3.47$; $df = 28$, $P = 0.002$). Both well-fed and unfed females crawled less when they were paired than when single (well-fed: paired median = 0.43, range = 0.20-0.61, single median = 0.56, range = 0.32-0.95; Wilcoxon Signed Ranks test, $z = 2.44$, $P = 0.01$, $N = 15$; unfed: paired median = 0.24, range = 0.13-0.54; single median = 0.38, range = 0.10-0.62; $z = 1.93$, $P = 0.05$, $N = 15$). In the unfed treatment, females with fewer eggs were observed eating more frequently than females that had more eggs (well-fed females: $r_s = -0.39$, $P = 0.003$, $N = 56$). In the well-fed treatment, we found no significant correlation between egg load and eating frequency (unfed females: $r_s = -0.18$, $P = 0.18$, $N = 56$ females).

Food deprivation did not affect overall measures of mating behavior. Unfed and fed females did not differ in their mating duration (well fed females: 0.61 ± 0.07 , $N = 15$; unfed females: 0.64 ± 0.06 , $N = 15$; $t = 0.34$, $df = 28$, $P = 0.74$), mating frequency (well fed females: 0.27 ± 0.03 , $N = 15$; unfed females: 0.21 ± 0.03 , $N = 15$; $t = 1.26$, $df = 28$, $P = 0.22$) or total mating activity (well fed females: 0.45 ± 0.03 , $N = 15$; unfed females: 0.43 ± 0.03 , $N = 15$; $t = 0.28$, $df = 28$, $P = 0.78$).

Female body size and egg load effect

Interestingly, the direction of the relationship between female body size and mating frequency differed for well-fed and unfed females (Figure 1a, 1b). In the well-fed treatment, larger females mated more frequently than smaller females, whereas in the unfed treatment, smaller females had a higher mating frequency than larger females (Table 1). We found no relationship between body size and either mating duration or total mating activity (Table 1). In the well-fed treatment, females with higher egg loads had higher mating frequencies (Table 1), but this pattern did not occur for unfed females (Table 1). Egg load was not related to mating duration or total mating activity (Table 1).

DISCUSSION

Our food deprivation treatment had the desired effect. As expected, females that had been deprived of food spent more time eating and moved less frequently than the group of well-fed females. However, we found no overall effect of food deprivation on female mating behavior; the mating frequency, duration, and total mating activity were similar between treatments. Females with fewer eggs ate more frequently, at least in the unfed group, but this increase in time devoted to feeding also did not affect female mating activity.

There are at least two explanations for the lack of a significant effect of female condition on her mating behavior. First, males may not be able to mate more readily even though females are less mobile. Mating behavior may instead be affected by density of beetles or the male's condition (Saeki et al. 2005c; Switzer et al., 2001), and not by female behavior.

Second, food deprivation may affect mating behavior, but the effects are not consistent among females with different intrinsic characteristics such as body size and egg load. Previous studies have shown that the interaction of multiple costs and benefits may lead to alternative mating strategies within species and populations in which females can either maximize the benefits or minimize the costs of mating (Arnqvist et al., 2004; Wedell et al., 2002). In our study, the costs and benefits of mating may vary from female to female depending on many factors such as condition, fecundity and body size. In support of this possibility, we found that female body size, together with her feeding history, had an effect on female mating frequency: well-fed, relatively large females and unfed, relatively small females mated more frequently than well-fed, relatively small females and unfed, relatively large females.

Food deprivation may have had a different effect on females of different sizes because metabolic costs per unit mass decrease with body size (Peters 1983). Thus, bigger females may be able to withstand food shortages better than smaller females (Provencher & Riechert, 1991). Because mating multiple times has both costs (Chapman, 1995; Daly 1978) and benefits (e.g. Boggs & Gilbert 1979; Fox, 1993; Ridley 1988; Simmons, 1989), perhaps females are minimizing the costs, by mating at low rates, or maximizing the benefits, by mating several times, depending on her condition. For example, smaller females that are strongly affected by food restriction could be maximizing the benefits of multiple matings by receiving water and/or nutritional substances from the ejaculate. In many insect species, males provide nutrient benefits to females in their ejaculate (Boggs,

1990), although the existence of such benefits have not been examined in Japanese beetles. On the other hand, the change in mating frequency may not be a result of selection on females; females of different size and feeding status may change their behavior in some manner that “unintentionally” makes them more or less available to males, while receiving no benefit from this change in behavior.

We also found that egg load had an effect on the female mating frequency, but again, that effect was dependent on her feeding experience. As egg load increased, mating frequency increased, but only for the well-fed treatment. More fecund females may be closer to oviposition and also mate more frequently to get more and newer sperm (Woodhead 1981; Wedell & Cook 1999). When females leave the food plants to oviposit, they may be without food for several days (Fleming, 1972). Thus, egg load may interact with a female’s energy levels to determine if she is ready to leave the food plants and then, if they need to mate or not. Similarly, for the unfed females, a lack of effect of egg load on mating frequency could occur because unfed females are not ready to leave the food plants, regardless of their fecundity status. Alternatively, the “decision” to mate may be driven by males, and males may be able to assess a female’s feeding history and/or her egg load (Saeki et al. 2005b) and adjust their behavior accordingly.

In conclusion, we found that a female may be more or less likely to mate depending on a linkage between her condition, body size and egg load. These patterns have several implications. First, because these female characteristics affected mating frequency, it is possible that variation among females is responsible for the temporal changes in mating

behavior observed on the field (e.g. Switzer et al. 2001, 2004, Kruse and Switzer 2007). Further studies comparing female intrinsic characteristics at different times of the day would contribute to a better understanding of the Japanese beetles mating dynamics on the food plants. Second, although our results indicated that the number of times a female mated was affected by her body size and egg load, these effects were dependent on her condition. Studies have shown condition dependence to be a major source of within-population variation in the expression of costly sexual traits (Hunt et al. 2005; Penton-Voak, 2003). Similarly, our results suggest that condition-dependence may be responsible for considerable variation in female mating frequency within a population and thus different intensities of sperm competition among female phenotypes. Finally, our results suggest that researchers need to be careful in interpreting patterns (or, in our case, the lack patterns) in female mating behavior. For example, if a study fails to find a hunger effect on female mating behavior, it may be that intrinsic characteristics interact with condition to shape mating behavior. In our study, our overall analysis between feeding history and mating behavior yielded no significant connection; it was only upon examining patterns between body size, egg load, and feeding history that the connections were apparent.

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FIGURE LEGENDS

Figure 1. Relationship between female mating frequency (number of different pairs during the 12 observation periods) with female body size for (a) well-fed ($r_s = 0.29$, $P = 0.023$, $N = 59$) and (b) unfed females ($r_s = -0.35$, $P = 0.008$, $N = 58$). We defined pairs to be “different” when the female was observed single for at least one observation period between consecutive pairs, even if the male with whom she was paired was identical in these pairs.

Fig. 1

Table 1. Relationships (Spearman r_s) between mating behavior and the body size or egg loads of well-fed ($N = 59$) and unfed ($N = 58$) female Japanese beetles.

		Mating behavior		
		Mating Frequency	Mating duration	Total mating activity
Well-fed females	Body size	0.29*	-0.065	0.14
	Egg load	0.27*	-0.15	0.06
Unfed females	Body size	-0.35**	0.02	-0.07
	Egg load	0.14	0.21	-0.09

* $P < 0.05$; ** $P < 0.01$.