Male Preference for Large Females and Female Reproductive Condition in the Japanese Beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae)

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**Abstract.** In the field, paired (in copula) female Japanese beetles (*Popillia japonica* Newman) tend to be larger than unpaired females. In this study, we investigated whether this size pattern could be explained by a male preference for large females, and whether larger females tended to have more and/or larger eggs than smaller females. In a laboratory study, both small and large males, when given a choice of a large and a small female, tended to choose the large female. We classified field-caught paired and unpaired females, measured their body size, and counted and measured their eggs. Larger females tended to have more and larger eggs than smaller females, indicating that males may benefit from choosing larger females due to the egg characteristics of these larger females. Paired females on the field were consistently larger than single females; paired females also had more and larger eggs, even when body weight was statistically controlled. Thus, although body weight of the female correlates with her fecundity, males may either use cues in addition to a female’s body weight to determine her immediate fecundity or fecund females may be more available for or less resistant to male mating attempts.

**Key Words.** Japanese beetles, Coleoptera, Scarabaeidae, body size, male mate choice, fecundity

Male mate preference for larger females has been observed in many insect species (reviewed in Thornhill and Alcock, 1983; Bondaransky, 2001). The function of this preference may have to do with egg production. Ward (1983) suggested that larger females may have more energy to produce eggs and the correlation between female size and fecundity has been documented in many studies (reviewed in Fox and Cressak, 2000). Overall, one might expect male mate choice when male investment is high, when the variation in female quality is high, and when searching and assessment of a mate are not costly (Thornhill and Alcock, 1983; Bondaransky, 2001; Simmons, 2001).

In this study, we examine male mate choice in the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). Japanese beetles have been recognized as agricultural and horticultural pests ever since they were accidentally introduced to the United States in 1916 (Fleming, 1972; Potter and Held, 2002). Their mating system is promiscuous and males exhibit post‐copulatory mate guarding (Fleming, 1972; Barrows and Gehr, 1978, Potter and Held, 2002). Virgin females emit a sex pheromone that attracts males (Ladd, 1976; Potter and Held, 2002); males and females subsequently mate on food plants (Fleming, 1972; Switzer et al., 2001). On food plants, mating pairs are more frequent in the morning and evening and the sex ratio is male biased (Switzer et al., 2001). No evidence currently exists for choice of males by females; however, contests among males for females are common and paired males are larger than unpaired males during some times of the day (Fleming, 1972; Switzer et al., 2001).

Switzer et al. (2001) found that paired females tended to be larger than unpaired females is the field and suggested that one possible explanation was a male preference for larger females. For Japanese beetles, mating may be energetically expensive for males because of high male-male competition for mates, and because males may guard their mate.
for hours after the copulation (Bottoms and Goeth, 1976). The time expended, predation risk, and energy consumed by the pairing male may result in male mate choice (Shelley, 1992; Hirzel et al., 1994). In this paper, we investigated male mate choice in the fish and also examined whether female body size was correlated with current egg number and egg size.

Materials and Methods

Male Choice Experiment

We conducted a male-choice experiment to examine whether males would prefer larger females. Beesles were collected randomly, without regard to pairing status, from four streams in the morning (primarily nonbreeder lotphotus albidus (Neff) Nes (Lotphotus), pool Pomus perspicillata (L.) Barrau (Sorexaceae) and spring, Glossoptus nauta (L.) Moer (Polynemaee) and brought to the laboratory for testing the day. Males were distinguished from females by size: of closely morphological (Barrau and Shelley, 1992; and large to small) individuals of each sex were selected from the collected population by eye. To test if male size affected their mate choice preference, we established large- (mean maximum body width = 6.07 ± 0.31 mm, 31 males) and small- (5.15 ± 0.20 mm, 50 males) treatments. In each trial, a male was placed with two females, one relatively large (5.35 ± 0.20 mm) and one relatively small (5.18 ± 0.20 mm) with a mean size difference of 0.19 ± 0.20 mm. Three beesles were individually marked with small dots of white correction fluid (Paper Mate, Liquid Paper). To provide a uniform substrate, all three beesles were placed on a disk of filter paper that had been dipped in a Systemfast® black. They were then covered with an inverted petri dish bottom (24 mm × 10 mm) and their mating behavior was observed. A male's choice was defined when he successfully copulated. If a male had not initiated copulatory behavior within 15 minutes of the beginning of the trial, the trial was ended. The maximum body width coinciding the interior and of the elytra of all beesles was later determined, using a dissection scope. Maximum body width is correlated with other measures of body size and is a good estimate of the size of the individual (Van Tonningen et al., 2006).

Characteristics of Pre- and Copulated Females

Poised mating females were collected on sweetweed (Polygynous sp.) (Poaceae); females in Oak County, Illinois at 13:30 by CDF on 5 Jul 2001. The samples were taken to the lab, pinned females were separated from their males, and both paired and single females were separately preserved in 70% ethanol until dissection. Before the dissection, maximum body width was measured. The eggs were classified into "mature" and "immature" eggs and counted. Mature eggs were defined as those eggs that were well-formed oval in shape, and before the size range given by Fleming (1972); these eggs are clearly distinguishable from "immature" eggs. The eggs were measured by width and height; these two measures were used to designate volumes as an overall measure of egg size. Because of the oval shape of the eggs, egg volume was calculated as: V = 0.52 × Length × Width².

Analyses

We tested whether pairing status and/or female body size affected egg volume and/or egg number. The difference in body sizes between paired and single females was tested using a two-parameter 2-simple test because the data were not normally distributed.
We tested for a difference in the mature egg volume between paired and single females with ANCOVA. The dependent variable for this analysis was egg volume; the independent variable was pairing status (i.e., paired or single) and the covariate was female body size. To avoid pseudoreplication, the mean mature egg volume was used for each individual because many females had multiple mature eggs.

We also used ANCOVA to test for a difference in the mature egg number between paired and single females. Mature egg number were transformed into natural logarithm after 0.5 was added because many females had zero mature eggs. The dependent variable for this analysis was egg number, the independent variable was pairing status and the covariate was female body size. Although the residual distribution violated the assumptions for ANCOVA, we also used Spearman correlations between body size and egg number, a non-parametric 2-sample t-test of the mature egg number between paired and single females, and a non-parametric 2-sample t-test of the body size between paired and single females. In these tests, results and statistical significance were similar to that of the ANCOVA test; therefore, we present the results of the ANCOVA here. All statistical tests were performed on SPSS Version 12.0 (SPSS Inc., Chicago, IL). All means are presented ± SE.

Results

Male Choice Experiment

In the male choice trials, 37/50 (74%) of the large male trials and 42/50 (84%) of the small male trials had a copulation within 15 min. For trials in which a copulation occurred, both large males and small males were significantly more likely to copulate with the large female (Fig. 1; large males: $\chi^2 = 9.7, \text{d.f.} = 1, P = 0.002 > P > 0.005$; small males: $\chi^2 = 4.66, \text{d.f.} = 1, 0.05 > P > 0.025$). Large males did not differ significantly from small males in their preference for large females ($\chi^2 = 0.77, \text{d.f.} = 1, P = 0.33$). No significant differences were found between trials in which the male copulated with a small versus a large female for male size, female size, male size relative to the small or large female, or female sizes relative to each other (Table 1).
Table 1. Comparison of male, female, and male plus female body sizes of male, female, and male plus female body sizes of A. nigripennis with 106 specimens of M. nitidula and 106 specimens of B. grisea

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
<th>Male + Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large</td>
<td>Small</td>
<td>P</td>
</tr>
<tr>
<td>Male</td>
<td>15.1 ± 0.06</td>
<td>11.9 ± 0.09</td>
<td>0.22</td>
</tr>
<tr>
<td>Large female</td>
<td>12.5 ± 0.03</td>
<td>10.2 ± 0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Small male</td>
<td>11.3 ± 0.03</td>
<td>8.6 ± 0.05</td>
<td>0.36</td>
</tr>
<tr>
<td>Small female</td>
<td>11.1 ± 0.02</td>
<td>8.4 ± 0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Male + female</td>
<td>21.6 ± 0.07</td>
<td>18.7 ± 0.05</td>
<td>0.15</td>
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</table>

Characteristics of Fiend-Captured Females

The body sizes of pairs of captured females were significantly larger than the single females (paired r = 0.29 + 0.035 mm, n = 80; single r = 0.29 ± 0.064 mm, n = 40; P = 0.04). Paired females had significantly more mature eggs (ANCOVA: F = 0.285, x2 = 0.034, P = 0.10). The effect of the covariate, body width, sex mature egg number was significant (Fig. 2A: ANCOVA: F = 0.44, B = 0.068, P = 0.02). In addition, paired females had significantly larger mature eggs (ANCOVA: F = 0.937, x2 = 0.019, P = 0.02). The covariate, body width, was positively related to the mature egg volume (Fig. 2B: ANCOVA: F = 0.44, B = 0.032, P = 0.03). Therefore, paired females had more and larger mature eggs.

Discussion

The male choice experiment demonstrated that male Japanese beetles did prefer larger females. This finding is consistent with results of other species (McLain and Brown, 1997; Fairburn, 1988; Peatt, 1988; Sih and Kup, 1992; Peatt et al., 1995), and suggests that the pattern observed previously in the field—paired females were larger than single females (Switzer et al., 2001)—could be explained by males preferring to pair with large females.

Our dissection study shows that larger females had more and larger eggs. A positive relationship between size and egg number has been observed in many insect species (Fairburn, 1988; O’Neill and Sklar, 1990; McLain et al., 1996; Switzer, 1995; McTinley and Gooling, 2000; Steenbom et al., 2002). To our study, mature egg number varied widely (i.e., from 0 to 13). For males, choosing a female with no eggs would be problematic, especially in a species with a test male advantage, such as Japanese beetles (Ladd, 1966). A female with no eggs would likely have a longer period of time prior to oviposition and therefore more opportunities to mate with other males. If a male chooses such a female, the whole process of mating, including mate guarding, may be too
Fig. 2. Female body width and (A) number of mature eggs (within 24 hours) and (B) volume of mature eggs in Japanese beetle. Line represents a simple linear fit to the data and to the results of the ANCOVA presented in the text.

costly (if he remains with the female) or a waste of time and resources (if he leaves the female and she mates). Thus, male assessment of egg load may be especially important in species in which some females have no eggs and in which there is a last male advantage (Blandin, 2004) and Brocks, 1998.

The positive relationship between egg size and maternal body size we found has also been observed in other insects (reviewed in Fox and Crisak, 2000). Thornhill and Alcock (1983) suggested that larger eggs are advantageous in terms of larval competition after hatching; for example, larger larvae may come from larger eggs (Fischer et al., 2002). Other studies showed additional advantages of larger eggs, such as greater embryonic viability (e.g., Arcevedo et al., 1997), larger larval mass size (e.g., Wallinhouse et al., 2001), higher larval survival rate (e.g., Solbreck et al., 1999), and faster growth rate (e.g., McLain
and Mallard, 1991). However, larger eggs may be disadvantageous due to a higher demand for oxygen (Kraak and Blaak, 1998).

Overall, our results suggest that a male pairing with a large female will receive benefits in terms of both the number and size of eggs fertilized. Of course, our dissection study could only determine the instantaneous fitness of a female, not her lifetime fitness. However, for species with multiple matings by females and a bimale advantage, intraspecific intramale rivalry is likely to be the most valid concern, as female eggs are likely to be fertilized by other males.

Interestingly, the results of our ANCOVA, which included factors with more and larger eggs, are more likely to be biased, especially when the effort of body size/ovarian weight of eggs and egg sizes were statistically eliminated. This suggests that although body width of the female seems to play a major role for the dissemination of the females, males may also be able to differentiate a female's fecundity with other morphological cues, such as thickness of the abdomen, or from a female's behavior. For example, females that are closer to oviposition may be more receptive for mating, rather than in terms of their location, by being less resistant to male mating attempts, or even by soliciting males. Nonetheless, these methods do not need to be mutually exclusive, and indeed may have synergistic effects. Further studies examining differences in the behavior of more and less fecund females would be helpful in this regard.

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Literature Cited


