# Little Evidence for Male Mate Choice for Female Size in the Bean Beetle (*Callosobruchus maculatus*)

Gwen Kirschke, Emily Smith, and Clara Drummond

Female size is an indicator of quality in insects because larger females often have higher fecundity. Therefore, males should prefer to mate with larger females due to their ability to produce many high quality offspring. However, mating with females much larger than a male's own size can be difficult, especially in a system where optimal mating frequency differs between the sexes and females can resist mating. This study examines male mate choice based on categories of relative female size (large/small) in the bean beetle *Callosobruchus maculatus*, as well as spermatophore investment in the chosen mate. We presented virgin male bean beetles with a choice of two virgin females that differed in size and predicted that males would attempt to mate with larger females more often than smaller females. Additionally, we predicted that males would transfer larger spermatophores during successful matings with the larger females. Although males were predicted to prefer mating with larger females, we expected that the male would have the most success mating with females closer to his own size, as females will kick to remove males attempting to mount them. Our results show that the number of mountings (a proxy for male preference), did not differ by female size category, but that males mated with smaller females slightly more often than expected. The male's spermatophore investment did not differ with the size of the female. This suggests that males might mate differentially based on female size, but not necessarily in accordance with our predictions.

Mate choice is a crucial aspect of evolutionary strategy in the animal kingdom and can be influenced by many characteristics that contribute to an individual's fitness such as age, size, fecundity, and ornamentation. It is widely accepted that the size of female insects can be an indicator of quality because larger females often have higher fecundity [1]. Additionally, male insects often invest in a mate through the transfer of resources such as in a spermatophore (a capsule of sperm and nutrients), so it has been implied that males should preferentially invest in larger rather than smaller females due to the larger female's ability to produce many high quality offspring [2, 3]. However, mating with females much larger than a male's own size may be challenging due to physical constraints.

The effects of female and male size on a female's decision to mate and re-mate has been studied in the bean beetle, *Callosobruchus maculatus* [1]. However, it is not clear from the existing literature how female size influences male mate choice in the bean beetle. Therefore, in our experiments we aimed to understand whether male bean beetles make mate choices based on female size. We tested male mate choice by running mating trials between males and females of varying sizes and measuring spermatophore investment and number of mountings as proxies for male preference. We predicted that (1) males would attempt to mate with (mount, climbing atop the female) larger females more often than smaller females. We also expected that (2) in successful copulations, males would invest larger spermatophores in females that were comparatively larger than themselves. Although we predicted that males would show a mating preference for larger females, we expected that (3) males would have the most success mating with females closer to their own size.

## Methods

*Obtaining Beetles*

Bean beetles (*C. maculatus*) were obtained from Carolina Biological, and propagated on organic mung beans (*Vigna radiata*) for two generations prior to experiments. The cultures were kept at 30°C with 40% relative humidity on a 12:12 light-dark cycle. We isolated virgins by placing beans with single windows (transparent circles on the surface of the bean indicating a contained pupa) into wells of a 96-well plate, the top of which was sealed with a breathable membrane. Windowed beans were separated by size of bean to isolate large and small beetles. These beetles were left for two days after emergence before use to allow time for the males to produce a spermatophore.

*Trials*

Beetles were removed from the 96-well plates and placed into individual petri dishes using featherweight forceps. We determined their sex based on dimorphic abdomen shape and color, and the females were sorted into large and small categories based on visual inspections of body size. Prior to each trial one person individually weighed one female from the large category, one female from the small category, and one male. The same 60mm petri dish was used to contain the beetles while they were being weighed, after which they were returned to their individual dishes. We confirmed by relative mass that one female in each trial was in fact lighter than the other female. If the females had the same mass, a different pairing was created. Either the large or small female, alternating between trials, was marked on their elytra (a protective covering for the wings) using nail polish (L.A. COLORS Color Craze #516).

After we provided at least one minute for the nail polish to dry, the two females were transferred to the mating dish (a 100mm petri dish), followed by the male (Figure 1 a). We started the trial as soon as the male was added. A single other observer watched each of the trials and noted the number of times the male climbed on top of each female (mountings), as well as the female in the first penetrative mating (Figure 1 b). The mating was observed until complete, after which the male was immediately removed and weighed again. If the male did not successfully mate within fifteen minutes, the trial was considered failed and the data were not included in the analysis.

All petri dishes were rinsed with tap water and dried before being used again. Forty-one trials were conducted.
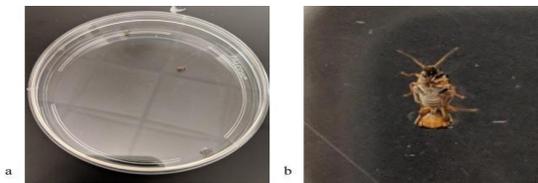


Figure 1. a.) Example mating trail. 100 mm petri dish containing (from top to bottom) a male, a marked female, and an unmarked female. b.) Example of a successful copulation. Beetles are viewed from below; male aedeagus (reproductive organ) is visibly in the female.

*Statistics*

We calculated the male spermatophore size by subtracting the post-mating male mass from the pre-mating male mass. This spermatophore size was then divided by the pre-mating male mass to determine the investment relative to male size.

We used a chi square test to examine whether female size category affected how many times that female was mounted. A chi square test was also used to examine whether female size category affected whether the male mated with her. This test was repeated for subsets of the trials, occurring at different times of day and on different days. Additionally, we used a chi square test to check if marking affected male mate choice. A logistic regression was used to see whether continuous female mass predicted male mate choice. A square root transformation was applied to the change in male mass and to the relative male investment to achieve normality, which were then used in a linear regression with chosen female mass. All statistics were done in R (R core team, 2018).

*Results*

There were thirty-six successful mating trials. The number of mountings did not significantly differ between the female size categories ($X^2 = 9.59$, $p = 0.0878$).

Males mated with the smaller female slightly more often than expected ($X^2 = 3.56$, $p = 0.0594$), as shown in Figure 2. The chi square tests run on subsets of the trials (e.g., time of day, day of trial) demonstrated that mating preferences did differ by size in one lab session (session one $X^2 = 0.133$, $p = 0.715$, $n = 18$; session two $X^2 = 1$, $p = 0.317$, $n = 12$; session three $X^2 = 7.54$, $p = 0.00604$, $n = 20$), and for trials run at different times of day (day $X^2 = 0.133$, $p = 0.715$, $n = 18$; night $X^2 = 7.71$, $p = 0.00548$, $n = 32$), and date (day one $X^2 = 0.0870$, $p = 0.768$, $n = 30$; day two $X^2 = 7.54$, $p = 0.00604$, $n = 20$). In all of these cases the males mated with smaller females more often. The proportions separated by date are shown in Figure 3.

Male mate choice of marked and unmarked females did not differ from expected. ($X^2 = 2$, $p = 0.157$), and female mass did not predict male mate choice ($p = 0.473$).

Larger spermatophores were not allocated to larger females ($p = 0.287$, adjusted $R^2 = 0.00591$), nor was male investment scaled for the male's size ($p = 0.315$, adjusted $R^2 = 0.001583$).
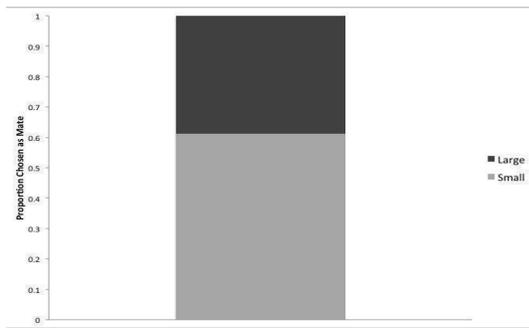
Figure 2. The proportion of small females mated with by males (grey) in relation to the proportion of large females mated with by males (black). Marginally more males mated with small females than they did with large females, $p = 0.0594$ and $n = 36$.
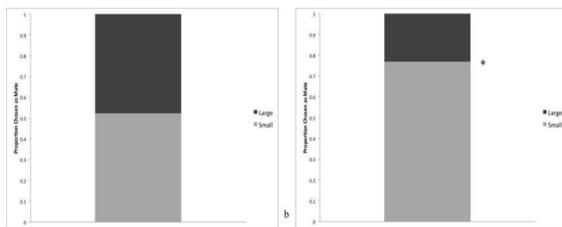


Figure 3. a.) The proportion of small females mated with by males (grey) on day one (7 Feb. 2019) in relation to the proportion of large females mated with by males (black) on day one. These did not differ, $p = 0.768$ and $n = 23$. b.) The proportion of small females mated with by males (grey) on day two (9 Feb. 2019) in relation to the proportion of large females mated with by males (black) on day two. Males mated with the smaller female more often, $p = 0.00604$ and $n = 13$.

## Discussion

Our data did not support our hypothesis that males prefer to mate with larger females, since mountings were not preferentially allocated to larger females. However, through our observations, we concluded that mountings may not have been the ideal indicator of male preference. In the majority of our trials, the only mounting that occurred was during the successful mating and not on occasions where mating did not occur. Anecdotally, we observed that in several instances the male actively pursued the larger female, but was unable to catch her and later mated with the smaller female, which is a type of behavior which would be predicted by our hypothesis, but more difficult to rigorously quantify (G. Kirschke, pers. obs.).

There was some support for our hypothesis that males would most successfully mate with smaller females, as across all of our trials males were mar-ginally more likely to mate with the smaller of the two females provided. However, this marginal preference was not consistent across our experimental sessions. Two of these sessions were conducted on the same day, with one in the afternoon and the other in the evening. The third session was conducted in the evening two days later. When the trials were divided by session, male mate choice did not differ from expected for the first two sessions, but males did choose small females more than expected in the third session. When trials were divided by the time of day, male mate choice did not differ from expected for the day time session, but males did choose small females more than expected in the night time sessions. Finally, when the trials were divided by date, male mate choice did not differ from expected the sessions on the first day, but males did choose the small females more than expected on the second day.

Since some of the sessions differed by time of day, circadian mating rhythms may have been a factor. A study done in cabbage beetles (*Colaphellus bowringi*) raised on a 12:12 light-dark cycle found a majority of matings occurring during light hours, with peaks at 8:00 and 16:00 [4]. However, since our night time trials on the first day did not show a significant difference from expected while the night time session on the second day did, we suspect that the difference was between the dates, and not the time of day.

It is unlikely that the date itself had any effect since the sessions were only two days apart. The beetles used on the two days did differ in two ways. The beetles used on day one were from windowed beans isolated 2-3 days earlier, while the beetles used on day two were from windowed beans picked 3-4 days earlier. Thus, the beetles used on day one were both younger and from earlier-laid eggs than the beetles used on day two. Since we did not select beetles of the exact same age each day, these variables are potentially confounded. Mating behavior has been shown to differ with male age in the ladybird beetle species *Coccinella septempunctata* L. and *Coccinella transversalis* Fab. [5]. We are not aware of any studies examining the mating behavior or general quality of beetles from later-laid eggs, but later-laid eggs likely come from females with fewer remaining resources and may have been laid on less desirable beans, resulting in lower quality offspring driven to mate quickly with whichever female they could catch. This is an interesting question that should be pursued in future research.

Another study found support for male mate

choice based on female sexual maturity in the flour beetle *Tribolium castaneum* [6]. Our procedure was designed to allow all beetles time to reach sexual maturity before being removed from the 96-well plates for use in trials, but while we were picking beetles we did notice some beans from which beetles had not emerged, and others emerging while we were picking, so we cannot be sure of their sexual maturity in any session. Thus, the mate choice observed could have been based on a combination of size and maturity.

Finally, we did not find support for increased spermatophore investment in larger females, either through the transfer of larger spermatophores or through the investment of a larger percentage of the male's mass. Spermatophore size generally decreases with each mating [7]. It may be advantageous for males to allocate their largest spermatophore to the first female they are able to mate with, regardless of her size, since their current mating is a guaranteed chance to transfer sperm, and the probability of getting a later mating with a larger female is relatively low.

Our results suggest that males may mate differentially based on female size, but our study was limited by confounded variables (age of beetles, time of day) and a relatively small sample size, further divided by session in some of the analyses. Moving forward, a different measure of preference than mounting attempts, or a combination of measures, should be used. Selecting beetles of the exact same age for trials over several different days could isolate the impact on their mating behavior of the time of the beetles' emergence relative to the peak, and an increased sample size (e.g., >75 trials) would allow for more reliable analyses for subsets of the data.

**Acknowledgements**

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