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Environmental Conditions Affect Sperm Competition Risk in Japanese Beetles (Coleoptera: Scarabaeidae)

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ABSTRACT Males of many species guard their mates to prevent rivals from usurping paternity of the potential offspring. Environmental conditions, such as temperature, may affect a male's ability to guard a female effectively and consequently the amount of sperm competition that occurs. We tested whether temperature and light affected mating behavior in laboratory experiments on the Japanese beetle, *Popillia japonica* Newman, a species in which males guard females for minutes to many hours after mating. When tested in groups, under conditions of high temperature and high light, males guarded females for shorter periods of time and males and females both mated with more different individuals. The number of mating pairs observed at any one time, however, was lower under conditions of relatively high temperature and light, which agrees with field data and is likely due to a combination of shorter pair durations and a period of time between successive mates. Contests for females were rare, which indicates that these patterns were due to changes in the behavior of the male and/or female of the pair. To tease apart male versus female roles in these patterns, single males were given dead females for copulation and guarding. When single males mated with the dead female, light and temperature still affected guarding behavior (e.g., the shortest durations were under conditions of relatively high temperature and light), indicating that males play a major part in determining the duration of mate guarding, probably due to a change in the costs the males are incurring. However, the patterns in the dead female experiment were not identical to the patterns in the group experiment, implying that female behavior also plays a role in guarding time. Our results demonstrate that environmental conditions can have a major effect on sperm competition risk and suggest that males would benefit greatly from being able to mitigate, either morphologically, physiologically, or behaviorally, the impact of those conditions.

KEY WORDS mating behavior, mate guarding, sperm competition, temperature, light

In species in which females mate with multiple males, fertilization is internal, females can store sperm, and there is a delay between copulation and fertilization, a male is not guaranteed to sire offspring simply because he transferred sperm to the female (Simmons 2001). In such species, sperm competition—the competition for paternity among the sperm of males simultaneously present within a female—can be an important selective force (Parker 1970). Consequently, evolution has led to morphological, physiological, and behavioral characteristics of males that increase the likelihood that their sperm will be used to fertilize the female's ova (reviewed in Simmons 2001). One of the most commonly observed methods used by males is postcopulatory “mate-guarding”; that is, physically preventing other males from mating with his female before fertilization (Parker 1974, Alcock 1994).

Mate guarding is particularly common among arthropods, and many studies have examined what characteristics of the male, female, or their social environ-

ment affect a male's guarding duration, and potentially his ability to guard successfully. Such studies have indicated that depending on the species, factors such as male size (Brown and Stanford 1992) and energy level (Sih et al. 1990), female size (Knox and Scott 2006), female breeding condition (Sillen-Tullberg 1981), sex ratio (Arnqvist 1992), and density of competitors (Sherman 1983, Stoks et al. 1997) all may impact how long a male guards a female. In some cases, it is clear that the particular characteristic may affect a male's likelihood of paternity. For example, smaller male thrips [*Elaphrothrips tuberculatus* (Hood)] are more likely to lose their female to a larger rival (Crespi 1989). In other cases, the male may be balancing how long he stays with a particular female relative to the net benefit he will achieve from guarding that female relative to other opportunities. In the carrion beetle *Necrophila americana* L., relatively large males guarded females longer when exposed to male-biased sex ratios (which increases the likelihood the female will encounter another male; Parker 1974) and when the guarded female was relatively large (Knox and Scott

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2006). Thus, trade-offs resulting from the state of the male or female can directly impact guarding, although in many cases it is difficult to distinguish whether the termination of guarding is a result of male or female behavior (Jabłoński and Vepsäläinen 1995).

In contrast to the individual and social characteristics listed above, studies have less frequently examined whether abiotic, environmental conditions can affect mate guarding (but see Singer 1987, Saeki et al. 2005b, Latty 2006), and fewer still have looked at the consequences of multiple mating and sperm competition. One obvious possible environmental condition is temperature; temperature has the potential to impact profoundly the extent and outcome of sperm competition in several ways. First, for ectotherms, temperature may directly impact the male's ability to effectively guard his mate. For instance, physiologically, temperature may affect a male's ability to fight off potential rivals or may increase the energetic or thermoregulatory costs for males (Singer 1987, Saeki et al. 2005b). Second, temperature may make the female more or less easy to guard. Females under extreme conditions may be less active and therefore easier for a male to protect. Alternately, temperature may change the activity of rivals, thereby affecting the amount of competition for any particular mate and the cost of mate guarding. Third, adverse conditions may make activities other than attempted usurpation of females a priority for males, their mates, or potential cuckolders. For example, in the bluethroat (*Luscinia s. svecica*), for those broods that had multiple paternity, mornings with relatively low temperatures were associated with a lower frequency of extra-pair paternity (Johnsen and Lifjeld 2003). Johnsen and Lifjeld (2003) suggested that this lower frequency was because individuals, who might otherwise be engaged in extra-pair copulations, needed to concentrate on foraging during such conditions.

In this study, we investigated the effects of environmental conditions, specifically temperature and light, on behaviors related to sperm competition in Japanese beetles, *Popillia japonica* Newman. Both male and female Japanese beetles are promiscuous, and there is a last-male advantage for fertilization (Fleming 1972; Ladd 1966; N. Tigreros and P.V.S., unpublished data). Japanese beetles have a scramble-competition mating system in which males search for virgin females via a female-released sex pheromone; response to this pheromone in the laboratory is dependent on light levels (Heath et al. 2001). Males also search for nonvirgin females (which no longer release the sex pheromone) on food plants (Fleming 1972). On food plants, beetles tend to aggregate (Fleming 1972, Iwabuchi and Takahashi 1983) and in these areas, males mount unpaired females and/or may attempt to usurp females from already paired males (Kruse and Switzer 2007; N. Tigreros and P.V.S., unpublished data).

After copulation with a female, the male may remain on her back and guard her for minutes to many hours (Barrows and Gordh 1978, Saeki et al. 2005b, 2005c; N. Tigreros and P.V.S., unpublished data). The

duration of this postcopulatory guarding may be longer under higher density conditions and if the female is relatively large (Saeki et al. 2005a). In the field, guarding males had higher thoracic temperatures (relative to ambient temperature) than unpaired males, and in laboratory studies, males guarded females for shorter durations under high temperature (Saeki et al. 2005b). The results of the laboratory study indicate that temperature may indeed affect a male's effectiveness in ensuring his paternity and that physiological constraints on guarding may at least partially explain the lower frequency of pairs found in the afternoon in field populations (Switzer et al. 2001). However, in the laboratory study of Saeki et al. (2005b), a single male and female were placed together in an environmental chamber under relatively low light. Thus, it remains unclear whether these results can be extended to more natural, multiple-beetle social situations (and hence to actual sperm competition), whether temperature can explain the variation in pair frequency observed in the field or if such patterns are better explained by variation in light levels, and to what extent the male versus the female is responsible for determining mate guarding durations. In this study, we addressed these possibilities in a laboratory study in which we observed pairing behavior in groups of beetles under differing levels of temperature and light.

Materials and Methods

The experiments described below took place during July 2006 by using beetles captured on food plants in Coles Co., IL. Before experiments, beetles were sexed using characteristic foreleg morphology (Smith and Hadley 1926). For some experiments, the size of the beetle was determined by using a dissecting microscope with an ocular micrometer to measure maximum body width (a measure that is a good estimate of body size due to positive correlations with other measures of body size; Van Timmeren et al. 2000).

Group Experiment. For testing the effect of temperature and light on the mating behavior of groups of beetles, we used mating arenas that consisted of acrylic boxes (16 by 16 by 16 cm) with air holes in the lid. Each box had a piece of white paper on the bottom, a sassafras leaf [*Sassafras albidum* (Nutt.) Nees] for food, and a small vial of water with a cotton wick to provide moisture. The sassafras leaf was taped to an index card to keep it flat (to facilitate observations of beetles); fresh leaves were provided as necessary during the experiment.

The night before the experiment, males and females were individually marked by gluing numbered bee tags (E.H. Thorne, Ltd., Wragby, United Kingdom) to their pronotum. For each trial, a random set of six males and four females was placed in each box and the box was placed in the environmental chamber at 18°C and no light. This sex ratio corresponds to the average sex ratio found on food plants (Switzer et al. 2001).

The morning of the experiment, light and temperature levels were gradually raised to reach treatment

conditions by 1000 hours. The four treatments were the four possible combinations of high temperature (HT, 32°C), low temperature (LT, 24°C), high light (HL, 3300 fc), and low light (LL, 800 fc). Light was provided by a combination of compact fluorescent and incandescent lights to approximate a daylight spectrum. Temperatures were verified with a thermocouple probe within the boxes (Omega Engineering, Inc., Stamford, CT), and light levels were verified with a hand-held light meter (Apogee Instruments, Inc., Logan, UT).

Starting at 1000 hours, the identity of the top and bottom individual of each pair was recorded every 15 min until 1600 hours. At 1600 hours, beetles from each box were bagged and frozen for later size measurements. Before the next trial, boxes were cleaned and the paper and leaves were replaced.

Analysis of Group Experiment. Homosexual pairs were relatively uncommon (only 13/240 males paired homosexually) and lasted relatively briefly (median = 1 period; $N = 17$ pairs) (see also Switzer et al. 2004); consequently, only heterosexual pairs were considered for these analyses. For analysis of pair frequency, we calculated the average number of pairs that existed over the 6-h observation period for each trial. For males, we calculated the number of separate pairs in which a male participated, the number of different females with which the male paired, the longest continuous pair for each male (i.e., number of consecutive observation periods), and the proportion of observation periods (of the 25 conducted over 6 h) in which a male was observed paired with a female. For females, we calculated the number of separate pairs in which she was involved, the number of different males with whom she paired, and the proportion of observation periods in which she was paired. Pairs were considered to be "separate" if the male and female from the pair were unpaired for at least one observation period between pairings, even if the subsequent pairing for the individuals was with the same partner. Treatments were compared with two factor analysis of variance (ANOVA). Sample sizes differ for some analyses because of some males or females losing their tags either during or after a trial. These and all other analyses were conducted with JMP statistical software, version 5.1.2 (SAS Institute, Cary, NC).

Activity Levels. In the group experiment (see above), we were only recording identities of paired individuals. To record how temperature and light affected activity levels, including the activity levels of unpaired individuals, we conducted a separate experiment in which we placed four males and six females in boxes as described for the group experiment. However, in this experiment, beetles were just marked as to male versus female (i.e., not marked individually) and, under the same four treatment conditions, we recorded activity levels of pairs, unpaired males, and unpaired females. Activity was noted as still, crawling, or flying. For this experiment, boxes were observed every 15 min from 1100 hours to 1300 hours.

Analysis of Activity Levels. Because few beetles flew during the observations, to analyze the activity data

we first combined the crawling and flying beetles into an "active" category. We then calculated the average proportion of inactive unpaired males, unpaired females, and pairs over the 2-h observation period for each trial. Using a proportion minimized the treatment effects on the absolute number of different individuals conducting those activities. We compared these proportions among treatments with a Kruskal-Wallis, nonparametric ANOVA.

Necrophilia Experiment. To distinguish the role of the male from that of the female in their guarding behavior, we took advantage of the fact that male Japanese beetles will copulate with, and subsequently guard, freshly dead females.

To test the effect of temperature and light on male behavior, we first captured females and separated them into large (7.06 ± 0.02 mm; $N = 119$; range, 6.8–7.68 mm) and small (6.15 ± 0.02 mm; $N = 114$; range, 5.44–6.52 mm) categories; this allowed us to also look for influences of female size on male guarding behavior. Females were then kept overnight in a freezer at -18°C . Before testing the next morning, females were removed and allowed to thaw. Once thawed, we inserted a #2 insect pin completely through the female's thorax from the right to the left side, until the head of the pin was contacting the right side of the beetle. The rest of the pin was then bent down at a right angle close to the beetle's body and the female was pinned to a foam board in a posture as life-like as possible. Pinning the female in this manner kept her secured and yet did not interfere with the male's mounting or copulation behavior. A piece of paper towel separated the beetle from the foam board and allowed us to control for any possible chemical cues between consecutive trials by simply replacing the paper towel.

For a trial, the female was placed under an inverted petri dish (24 mm in height; 90 mm in diameter) in the environmental chamber at treatment conditions. Likewise, a single male was placed in a separate petri dish in the chamber. Both beetles were given 15–30 min to acclimate to treatment conditions before testing. For testing, the male was placed with the female under her dish and the times associated with the following events were recorded: mounting the female, removal of the aedeagus (i.e., the male copulatory organ), and dismounting the female. Insertion of the aedeagus is more cryptic and was not recorded for this experiment; however, it typically occurs shortly after the male mounts the female (P.V.S. et al., unpublished data). If no copulation occurred in 10 min, the trial was recorded as "no copulation"; otherwise, the trial duration depended on the duration of postcopulatory guarding, with trials ending after 5 h of guarding. Only 11/233 trials needed to be terminated while males were still guarding (i.e., guarding continued for longer than 5 h for these 11 trials).

Analysis of Necrophilia Experiment. For those males that copulated with females, we calculated the latency until mount and the guarding duration (i.e., amount of time between aedeagus removal and dismount). These dependent variables were incorpo-

Fig. 1. Average number of heterosexual pairs ($\pm$ SE) in each box per observation period. HTHL, high temperature, high light; HTLL, high temperature, low light; LTHL, low temperature, high light; LTLL, low temperature, low light. The number above the bar represents the sample size (i.e., number of separate trials).

rated into a three factor ANOVA with the independent variables of temperature level, light level, and female size.

Results

Group Experiment. The number of pairs at any one time was affected by the environmental variables (Fig. 1). Pairs were less frequent with more light and higher temperatures (temperature: $F_{1,36} = 43.3$, $P < 0.0001$, light: $F_{1,36} = 5.75$, $P = 0.02$), and there was no significant interaction between the two ($F_{1,36} = 1.1$, $P = 0.31$).

When looking at individual males, light and temperature had affected their pairing behavior (Fig. 2). Under conditions of high temperature and/or high light, males paired more frequently (Fig. 2A; temperature: $F_{1,236} = 68.5$, $P < 0.0001$, light: $F_{1,236} = 13.1$, $P < 0.0004$), paired with more different females (Fig. 2B; temperature: $F_{1,236} = 56.4$, $P < 0.0001$, light: $F_{1,236} = 10.4$, $P = 0.0015$), and they had a shorter “longest continuous guard time” (Fig. 2C; temperature: $F_{1,236} =$

Fig. 2. Pairing behavior of male Japanese beetles as affected by temperature and light treatments: average ($\pm$ SE) number of pairs per male (A), average number of different females with whom a male paired (B), average longest continuous guarding duration (C), and proportion of all observation periods in which the male was paired (D). Sample sizes are HTHL = 72, HTLL = 60, LTHL = 54, and LTLL = 54 (abbreviations as in Fig. 1).

94.7, $P < 0.0001$, light: $F_{1,236} = 20.1$, $P < 0.0001$). Temperature, but not light levels, affected the total percentage of time males paired (Fig. 2D; temperature: $F_{1,236} = 77.0$, $P < 0.0001$, light: $F_{1,236} = 9.3$, $P = 0.004$), with males pairing for a lower percentage of the time at higher temperatures. No significant interactions existed between temperature and light for any of the above analyses. The number of males (out of six possible per box) that paired in each box decreased with increasing temperature but not light (HTHL = 5.8 ± 0.11 , $n = 12$; HTLL = 5.7 ± 0.15 , $n = 10$; LTHL = 5.2 ± 0.36 , $n = 9$; LTLL = 5.0 ± 0.23 , $n = 9$; temperature: $F_{1,36} = 8.81$, $P = 0.005$, light: $F_{1,36} = 0.65$, $P = 0.43$), with no significant interaction.

Light and temperature had similar effects on the pairing behavior of females as with males (Fig. 3). Under conditions of high temperature and/or high light, females paired more frequently (Fig. 3A; temperature: $F_{1,152} = 75.4$, $P < 0.0001$, light: $F_{1,152} = 18.4$, $P < 0.0001$), paired with more different males (Fig. 3B; temperature: $F_{1,152} = 64.5$, $P < 0.0001$, light: $F_{1,152} = 19.0$, $P < 0.0001$). Temperature, but not light levels, affected the total percentage of time females paired (Fig. 3C; temperature: $F_{1,152} = 48.7$, $P < 0.0001$, light: $F_{1,152} = 0.22$, $P = 0.64$), with females pairing for a lower percentage of the time at higher temperatures. As with males, no significant interactions existed between temperature and light for any of the above-mentioned analyses. Contests for females were rare; only five fights were recorded of 1,026 total observation periods for all trials.

Activity Levels. Paired and single beetles of both sexes were more likely to be inactive under low temperature conditions: pairs (HTHL: 0.61 ± 0.07 , $n = 7$; HTLL: 0.76 ± 0.05 , $n = 8$; LTHL: 0.95 ± 0.04 , $n = 8$; LTLL: 0.93 ± 0.03 , $n = 8$; Kruskal-Wallis $\chi^2 = 15.5$, $df = 3$, $P = 0.001$), single males (HTHL: 0.31 ± 0.06 , $n = 7$; HTLL: 0.37 ± 0.08 , $n = 8$; LTHL: 0.80 ± 0.05 , $n = 8$; LTLL: 0.74 ± 0.04 , $n = 8$; Kruskal-Wallis $\chi^2 = 21.5$, $df = 3$, $P < 0.0001$), and single females (HTHL: 0.12 ± 0.08 , $n = 7$; HTLL: 0.41 ± 0.12 , $n = 8$; LTHL: 0.94 ± 0.05 , $n = 6$; LTLL: 1.0 ± 0.0 , $n = 6$; Kruskal-Wallis $\chi^2 = 19.6$, $df = 3$, $P = 0.0002$).

Necrophilia Experiment. Males copulated with and guarded the dead females; overall, 140/233 (60%) of trials resulted in a copulation, and this frequency did not differ among treatments ($\chi^2 = 6.16$, $df = 3$, $P = 0.10$). Males presented with larger females mounted them more quickly than males presented with smaller females (overall mean latencies: large female = 275 ± 25.8 s, small female = 355 ± 25.8 s; $F_{1,132} = 5.00$, $P = 0.027$); no other variables were significantly associated with mount latency (all $P > 0.30$). Once copulation was over, the amount of time a male remained mounted on a female ("mate guarding") was affected by female size, light, and temperature, but the effects were complex (Fig. 4). Males guarded large females longer than small females ($F_{1,132} = 4.37$; $P = 0.038$), and a borderline significant interaction existed between female size and temperature ($F_{1,132} = 3.77$; $P = 0.054$), with small females at high temperature having much shorter guard times than the other three treat-

Fig. 3. Pairing behavior of female Japanese beetles as affected by temperature and light treatments: average ($\pm$ SE) number of pairs per female (A), average number of different males with whom a female paired (B), and proportion of all observation periods in which the female was paired (C). Sample sizes are HTHL = 48, HTLL = 40, LTHL = 32, LTLL = 32 (abbreviations as in Fig. 1).

Fig. 4. Average ($\pm$ SE) guarding duration of male Japanese beetles after copulation with large or small dead females under temperature and light treatments. Sample sizes are given above bars. Abbreviations are as in Fig. 1.

ments (1223 ± 177 s, $n = 33$; the other three treatments were all $>1,900$ s). Guard times also were affected by temperature and light, but the effect was not consistent. Higher temperatures tended to lead to shorter guard times ($F_{1,132} = 3.76$; $P = 0.054$), but there was no independent effect of light ($F_{1,132} = 0.32$; $P = 0.57$). However, a significant interaction existed between temperature and light, which indicates the differential effect of light on guard time ($F_{1,132} = 7.0$; $P = 0.009$). The longest guard times were at high temperature and low light and the shortest guard times were at high temperature and high light.

Discussion

Pair Frequency and Sperm Competition. In our group experiment, we found that temperature, and to a lesser extent light, had clear effects on the mate-guarding behavior of male Japanese beetles. Under conditions of high temperature and light, males guarded females for shorter periods of time. Thus, the results of this study are consistent with the results of the one male/one female study of Saeki et al. (2005b). However, because we were testing beetles in groups and under natural sex ratios, we were able to show that the consequence of these shorter guarding periods was an overall lower pair frequency under high temperature and light. This lower pair frequency occurred even though each male paired more times under those conditions; the lowered pair frequency at the group level, therefore, results from a combination of the shorter guarding times and a time lag between pairs. Our results suggest that it is likely, therefore, that the higher temperatures and light levels in the afternoon are at least partly responsible for the lowered male-

female pair frequency observed in the field (Switzer et al. 2001).

Most importantly, the patterns we observed in our group experiment suggest that temperature and light have the potential to affect dramatically the sperm competition that males experience. Under high temperature and high light, females had more mates. Thus, if the last male sperm precedence patterns found by Ladd (1966) for Japanese beetles hold for situations in which females have more than two mates, and our observed patterns are consistent with what occurs in the field (which seems likely; see Switzer et al. 2001), the consequence of our results is that environmental conditions can have significant effects on a male's certainty of paternity and result in a significant variation in paternity association on a daily scale.

These patterns have several interesting implications for selection pressures on males and females. First, if some males are better able to mitigate the effects of environmental conditions, then they could be at a selective advantage. For example, large males, due to a smaller surface area-to-volume ratio, may experience lower physiological stress (Peters 1983) and thus may be able to guard for longer. Second, to the extent that mate choice exists or is possible, males may be selected to adjust their preferences for mates in concert with environmental conditions. For example, under conditions of high temperature and high light, males would benefit from identifying females that are close to leaving for oviposition. Some support exists for this occurring in Japanese beetles, because paired females may have higher egg loads than unpaired females (Saeki et al. 2005a; N. Tigreros and P.V.S., unpublished data). Similarly, if males can adjust their sperm allocation in response to levels of sperm competition risk (Engqvist and Reinhold 2005), then males may alter their ejaculates according to the relative risk under different environmental conditions. Third, males may prefer to pair with females in locations or at times that allow them to maximize their ability to guard effectively. Thus, the pattern observed in the field (i.e., higher pair frequency in morning and evening; Switzer et al. 2001) could reflect both a change in males' ability to guard females and a change in males' motivation to mate. Fourth, females, by choosing locations that are cooler or warmer, or by choosing to visit mating locations at cooler or warmer times, may be able to influence the amount of sperm competition that occurs. Future work should focus on identifying whether these ideas are supported in Japanese beetles or in other species that experience similar environmental impacts.

The behavioral changes we observed in high light and high temperature beg the question of why these conditions cause the change in behavior. For males, higher temperature could have increased the physiological costs incurred from guarding (Saeki et al. 2005b); guarding males are unable to eat to replenish their energy and water supply because they are on the backs of females. Shorter guard times and higher pair turnover also could have been a result of increased physical contests for females; however, we observed

very few fights, which makes it unlikely that usurpation was a primary cause of pair turnover. In the field, contests for females were observed for 1–3% of pairs at any particular time (Kruse and Switzer 2007); the low number we observed in the laboratory (much <1%) suggests that conditions that lead to contests for females in the field may not have been replicated in our laboratory experiment. Finally, pair turnover also could be a consequence of the higher activity level we observed at high temperature. Females, because they are more active, may simply be more difficult for a male to grasp for extended periods.

Explaining the increase in number of mates under conditions of high light is more problematic. It is certainly possible that high light levels also serve to increase the body temperatures of the beetles, and thus the explanation for the light effect is the same as for temperature. The shiny body Japanese beetles (Fleming 1972) is such that reflection of light likely occurs, but the extent that this reflection helps them avoid increases in their body temperature has not been quantified. However, Saeki et al. (2005b) found that both paired and single beetles of both sexes had higher body temperatures relative to ambient in the sun compared with in the shade. Therefore, higher light levels may lead to increased body temperatures in the field, and this same effect may have occurred in the current laboratory experiment. Alternatively (or in addition), light may act as a proximate cue for mating activity. Light levels seem to affect mating activity in several species (Svrd and Wiklund 1988, Hirota and Obara 2000), and Heath et al. (2001) found that male Japanese beetles were more responsive to sex pheromone under conditions of higher light and temperature and speculated that light might affect how well a male can find a mate or a host plant.

Male Versus Female Role. In the *Necrophilia* experiment, the dead females were clearly unable to behaviorally influence the initiation of mating behavior or termination of mate guarding. The results of this experiment thus demonstrate that in Japanese beetles, the male plays a role in the termination of postcopulatory guarding. As in the group experiment, males guarding a dead female did so for a shorter period under conditions of high temperature and light. The patterns were not as straightforward as those in the group experiment, however, with the longest guarding times occurring at high temperature and low light. The significance of guarding longer under low light and high temperatures is unclear, but the difference between the *Necrophilia* experiment and the group experiment suggests that either the female does play a role in guarding duration (perhaps due to her activity levels; see above), or that the social environment influences guarding duration (Saeki et al. 2005c).

Finally, in the *Necrophilia* experiment, males mounted large females more quickly and guarded them for longer after copulation. These results are consistent with previous work that has shown that Japanese beetle males will copulate with large over small females when given a choice (Saeki et al. 2005a) and will guard large females for longer (Saeki et al.

2005b, but see Saeki et al. 2005c). The results of the current study, however, provide conclusive evidence for the role of the male in these size-patterns. The benefit for those patterns may be because larger females have more or larger eggs total or per clutch (Saeki et al. 2005c), because large individuals are more likely to be females as opposed to males (P.V.S. et al., unpublished data), or perhaps large females oviposit more frequently.

In conclusion, we found that environmental conditions affected mate guarding behavior, which in turn affected the sperm competition risk that a male would experience. These patterns are likely to be found in other largely ectothermic species, especially those for which, like Japanese beetles, mate guarding seems to compromise their ability to thermoregulate. Furthermore, even if temperature does not affect guarding behavior per se, it still may affect sperm competition risk if, for example, intruding or "sneaker" males are better able to regulate their body temperature than the territorial individuals. Studies that attempt to relate mating success to variation among individuals in their ability to thermoregulate would be especially useful to test these possibilities.

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