# Effects of Environmental and Social Conditions on Homosexual Pairing in the Japanese Beetle (*Popillia japonica* Newman)

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*Homosexual pairing between males occurs under natural conditions in a wide variety of taxa, including many insect species, but few studies have investigated how environmental and social conditions affect same-sex pairing in insects. We investigated factors affecting homosexual pairing in male Japanese beetles (*Popillia japonica *Newman) in the field and in the laboratory. Specifically, we investigated how time of day, sex ratio, beetle density, and temperature affected the likelihood of homosexual pairing. In the field, male–male pairs constituted 1–6% of the pairs we collected. Homosexual pairs were more common in the afternoon than in the morning and the evening. Sex ratio, density, and temperature were all related to the likelihood of finding a homosexual pair, but the relationships were not linear. In the laboratory, higher male densities and relatively male-biased sex ratios were associated with an increase in the frequency of homosexual pairs. Homosexual pairs were more frequent at relatively low and relatively high temperatures. Males that mounted other males tended to be smaller than the males that they mounted. In addition, compared to males that were not homosexually paired, there was some indication that the mounting males were smaller, and the mounted males larger, than the unpaired males. Our data suggest that homosexual pairs are a result of males mistaking other males for females, and we hypothesize that the environmental*

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*and social factors cause changes in homosexual pairing through their effects
on the frequency of pair formation and pair duration.*

## INTRODUCTION

Homosexual behavior has been observed in a variety of animals, including
nonhuman primates, birds and other reptiles, amphibians, and insects (e.g.,
Verrell and Donovan, 1991; Jennsen and Hovde, 1993; Leal *et al.*, 1998; Vasey
*et al.*, 1998; Yamane, 1999). Functional explanations for the behavior typi-
cally fall into three categories. First, in some species, homosexual behavior
is viewed as adaptive; for example, homosexual behavior in female Japanese
macaques may help serve to establish alliances between females. These al-
liances may benefit females in later aggressive interactions with other mem-
bers of the troop (Vasey, 1996). Harari and Brockmann (1999) suggested
that female weevils (*Diaprepes abbreviatus*) behaved sexually toward other
females in order to attract larger, perhaps higher-quality males. Second, in
other species, homosexual behavior is thought to be an artifact of artificial
captive conditions. For instance, both male rams and male house finches will
exhibit sexual behavior toward other males when housed without females
(Price *et al.*, 1988; McGraw and Hill, 1999). Finally, homosexual behavior in
many species, including many insects, has been explained as simply an er-
ror; males exhibit sexual behavior toward other males because they mistake
them for females (e.g., Wang *et al.*, 1996).

Although it has been observed in the wild for species in at least seven
orders of insects (e.g., Shah *et al.*, 1985; Okada *et al.*, 1990; Dunkle, 1991;
Iguchi, 1996; Oguma *et al.*, 1996; Vicidomini, 1997; Leal *et al.*, 1998), nat-
urally occurring homosexual behavior has rarely been studied in detail for
any insect species (but see Harari and Brockmann, 1999; Harari *et al.*, 2000;
Serrano *et al.*, 1991, 2000). Generally, authors mention that homosexual be-
havior occurs and then briefly explain it away as a mistake (e.g., Tokeshi and
Reinhardt, 1996). This lack of detailed investigation into naturally occur-
ring homosexual behavior is unfortunate for three reasons. First, studying
the characteristics of homosexual individuals can provide clues as to the
characteristics used to identify sexes and potentially give insight into the
competition between males for females. Second, little is known of the costs
and potential benefits of homosexual behavior in the wild for most species.
To begin to understand the costs and benefits of homosexual behavior, we
must first identify the characteristics that are associated with homosexual
pairing in the field. Third, if homosexual is a costly behavior that decreases

an individual's reproductive success, knowledge about homosexual behavior for pest species may lead to management strategies that help control populations with less pesticide use.

We studied male–male sexual behavior in the field and laboratory in the Japanese beetle, an exotic species that was introduced into the United States in the early 1900's (Fleming, 1972; Potter and Held, 2002). Their larvae feed on roots of grasses; the adults emerge over a 2- to 3-month period (Van Timmeren *et al.*, 2000; Potter and Held, 2002) and feed on the leaves and flowers of over 300 species of plants (Fleming, 1972). Virgin females emit a sex pheromone (Ladd, 1970; Tumlinson *et al.*, 1977), but they stop producing this pheromone after their first mating (Ladd, 1970), even though they may copulate and lay eggs repeatedly during their 1- to 2-month adult life span (Fleming, 1972; Van Timmeren *et al.*, 2000). Males and females aggregate on food plants in response to plant kairomones (Loughrin *et al.*, 1995). While the level of aggregation varies widely (Switzer *et al.*, 2001), Japanese beetles can often occur at very high densities (Fleming 1972). For example, in 2001 we captured 73 and 68 beetles on two adjacent peach fruits (the fruits had estimated surface areas of 63 and 45 cm$^2$, respectively). On these food plants, males mate with females and may continue to ride on the backs of females, guarding them from other males, for hours after mating (Fleming, 1972; Barrows and Gordh, 1978; Potter and Held, 2002). Both males and females may mate with multiple partners during the day.

In this study, we examined how time of season, time of day, density, temperature, and sex ratio affected existence of male–male pairs in the field. We then conducted laboratory experiments to investigate more specifically how density, temperature, and sex ratio affected male–male pairing.

## METHODS

### Field Studies

Initial field collections were conducted in late July and early August 1999 in Coles County, Illinois, USA (in the middle of the breeding season for this location), as part of a larger study on mating behavior in the Japanese beetle (Switzer *et al.*, 2001). Specifically, beetles were collected at 0700, 1000, 1300, 1600, and 1900 h on 13 days. All collections took place in the same soybean field on soybean plants; soybeans are a preferred food plant of Japanese beetles (Fleming, 1972). To minimize the effects of sampling without replacement, no two collections took place at the same location. At each sampling period, we collected 25 beetle pairs (recognized by one beetle being on top

of another beetle in a "mate guarding" position [Barrows and Gordh, 1978]) and 50 unpaired beetles. Additionally, we counted the number of pairs per 100 beetles and, as a measure of density, the area (to the nearest 0.25 m$^2$) that these 100 beetles occupied. We combined the number of pairs per 100 beetles with the sex ratio of the unpaired beetles to estimate the population sex ratio (Switzer *et al.*, 2001). We also recorded the ambient temperature; as expected, the temperature was lowest in the morning and evening (time, mean temperature [°C]: 0700, 24.1; 1000, 32.4; 1300, 33.7; 1600, 33.1; 1900, 27.2]. Sex (determined by the characteristics of the front forelegs [Smith and Hadley, 1926]) and size (maximum lateral body width, measured using a dissecting scope with an ocular micrometer; [Van Timmeren *et al.*, 2000]) were determined for all 4875 beetles that were collected. We had previously determined that maximum body width correlated highly with other measures of body size such as dry mass (Van Timmeren *et al.*, 2000) and that females, on average, are larger than males (male mean body width = 5.82 mm; female mean = 6.26 mm [Switzer *et al.*, 2001]).

For our initial field collections, the status (i.e., whether the individual was the top or bottom beetle of a pair) was not recorded. To examine characteristics of the individuals that were homosexually paired, for 3 consecutive years (2000, 2001, and 2002) we collected hundreds of pairs in the field, keeping the top and bottom beetle distinct. In the laboratory, these beetles were sexed (so that homosexual pairs could be identified) and measured. In addition, in 2001 (the year in which we captured the most homosexual pairs in the field), we simultaneously captured 50 heterosexual pairs and 50 unpaired males for comparison.

### Laboratory Experiments

Japanese beetles readily exhibit mating behavior in the laboratory (Barrows and Gordh, 1978; personal observation); this allowed us to conduct three laboratory experiments to independently examine the effects of density, sex ratio, and temperature on male–male pairing. To limit any effect of captivity, for each experiment, beetles were captured in the morning in the field on a variety of food plants (primarily sassafras, *Sassafras albidum*; peach *Prunus persica*; and soybean, *Glycine max*) and brought to the laboratory for testing that day. Beetles were captured without regard to mating status. Tests took place 3–9 h after capture and different treatments within an experiment were conducted simultaneously to eliminate the chance of time since capture biasing the results. A total of 5890 individuals was used in the experiments; each individual was randomly chosen for a particular experiment and used only once.

For the density experiment, we placed 20, 30, or 40 male beetles in a rectangular glass container (30 cm long × 15 cm wide × 20 cm high). On the bottom of the container we placed a moist paper towel and on the sides we used cellophane tape to attach six leaves from a preferred food plant (either cherry, *Prunus* spp. cultivar, or sassafras, *Sassafras albidum* [Fleming, 1972]) for a food source and climbing substrate; two leaves were placed on each long side of the container and one leaf was placed on each short side. Leaves were placed such that one end touched the bottom of the container and the other end was approximately halfway up the side of the container. Temperature was maintained at approximately 28°C. After 1 h, all paired beetles were captured, keeping the top and bottom beetle distinct, as well as all single beetles. All beetles were frozen for later measurement. We conducted 25 trials of each density treatment.

For the sex ratio experiment, we used 20 beetles and varied the number of males and females in six treatments: 20 M:0 F, 16 M:4 F, 12 M:8 F, 10 M:10 F, 8 M:12 F, and 4 M:16 F. In order to identify the sex of the beetle, we marked males and females using distinct marks of white photocopy correction fluid. These 20 beetles were placed in a circular glass dish (10-cm diameter, 4-cm height), with two food plant leaves (sassafras) taped to the side of the dish. Temperature was maintained at approximately 28°C. The number of homosexual and heterosexual pairs was recorded every 15 min for 1 h. We conducted 14 trials of each sex ratio treatment.

For the temperature experiment, we placed 20 males in dishes set up identically to those in the sex ratio experiment. An environmental chamber was used to run experimental trials at three constant temperatures: 23, 27, or 37°C. All males were kept in large groups at the temperature of their trial for 2–3 h prior to testing. Separate groups of 20 males were then placed in the environmental chamber and the number of homosexual pairs was recorded after 1 h. Approximately 30 trials were run for each treatment.

## Analyses

For our systematic field collections, we conducted a logistic regression analysis using JMP statistical software (SAS Institute, 1995), with presence of a male–male pair as the dependent variable and date, time period, temperature, density, and operational sex ratio as the independent variables. The effects of the independent variables on Japanese beetle sexual behavior were not linear (Switzer *et al.*, 2001; see Results); including the independent variables as nominal variables yielded the best fit, superior to alternative methods, including that of incorporating the square of the independent variables. To convert the independent variables to nominal variables, we used the

25% percentiles around the median to categorize the data (except for time period, which had five levels corresponding to the five collection periods). We examined the significance of the overall model using likelihood-ratio tests and then used chi-square tests on individual parameter estimates to examine how the various levels within a variable compared to each other (SAS Institute, 1995). These comparisons among levels necessarily use one of the levels as the benchmark for comparison; thus, we can only examine how the other levels compare to this benchmark level and the results are presented relative to that benchmark. For example, time period had five levels but only four comparisons are made among levels in Table II. Nonparametric statistics were used when the data did not conform to the assumptions of parametric statistics and took tied values into account when appropriate. Means are presented ±1 SE.

## RESULTS

### Homosexual Pairs in the Field

Male–male pairs were found in 30 of the 65 collection periods, with an average of $0.85 \pm 0.12$ male–male pairs (3.4%) of 25 collected pairs (range, 0–3 pairs). Time period, temperature, density, and sex ratio, but not date, were significantly related to finding a male–male pair in a given sample (Table I). Time period had the strongest effect (Table I), with the least male–male pairing in the morning and evening and the most in the late afternoon (Fig. 1, Table II). Temperature also had a nonlinear effect on male–male pairing. Compared to the highest temperature quartile, male–male pairs were more likely at lower temperatures and less likely at middle to high

**Table I.** Results of Logistic Regression for Field-Caught Japanese Beetles

| Variable[a] | df | $\chi^{2b}$ | P |
|---|---|---|---|
| Date | 1 | 2.1 | 0.15 |
| Time | 4 | 25.2 | <0.0001 |
| Temperature | 3 | 7.6 | 0.05 |
| Density | 3 | 9.4 | 0.03 |
| Sex ratio | 3 | 8.7 | 0.03 |

[a]The five independent variables are listed; dependent variable was the likelihood of homosexual pairing. Temperature, density, and sex ratio were included as nominal variables using their 25% percentiles around the median.
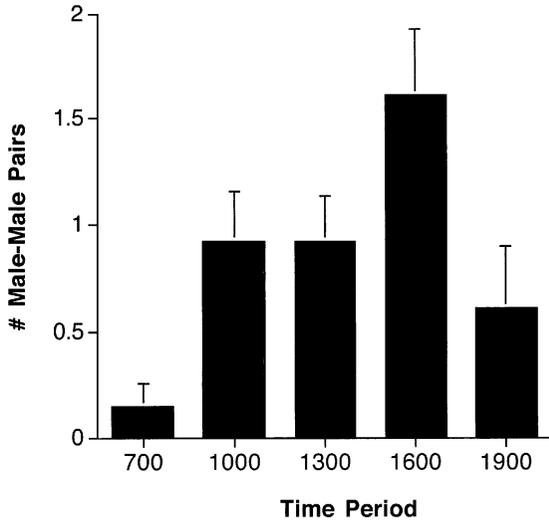[b]$\chi^2$ value and significance (P) values are based on likelihood-ratio tests.

**Fig. 1.** Average (±SE) number of male–male Japanese beetle pairs of the 25 pairs collected during each of the five sampling periods within a day ($N = 13$ sampling days for each time period).

temperatures (i.e., the 50–75% quartile; Table II). Therefore, the effects of time period and temperature did not directly correspond; e.g., the lowest temperatures (higher likelihood of male–male pairs) occurred during the 0700 time period (lowest likelihood of pairing), suggesting that temperature and period may have had partially separate effects on male–male pairing. In general, higher densities were related to a higher likelihood of male–male pairing (Table II), with the 50–75% quartile yielding a higher likelihood of male–male pairs than the highest density quartile. Finally, although sex ratio significantly affected the likelihood of finding a male–male pair (Table I), the pattern among sex ratio quartile levels was not intuitive. The only significant level was a lower likelihood of finding a male–male pair in the 50–75% quartile than at the most male-biased sex ratios. More female-biased sex ratios did not exhibit a lower likelihood of male–male pairing. Overall, these field collections indicate that several environmental and social factors were associated with the likelihood of male–male pairing, but while statistically significant, the relationships were not linear and the patterns were not intuitively obvious.

To examine characteristics of individual male–male pairs, we captured 23 male–male pairs in the field in 2000, 2001, and 2002, keeping the top and bottom beetles distinct. In 17 of 23 (74%) of the pairs, the top beetle was

**Table II.** Parameter Estimates for the Results of the Logistic Regression
Analysis for Japanese Beetle Homosexual Behavior

| Term[a,b] | Estimate (±SE)[c] | $\chi^2$ | $P$ |
|---|---|---|---|
| Time | | | |
| 0700–1900 | 5.9 ± 2.0 | 8.75 | 0.003 |
| 1000–1900 | −2.2 ± 1.0 | 4.95 | 0.026 |
| 1300–1900 | −2.3 ± 1.0 | 4.99 | 0.025 |
| 1600–1900 | −3.7 ± 1.3 | 8.41 | 0.003 |
| Temp | | | |
| 1–4 | −3.2 ± 1.5 | 4.38 | 0.036 |
| 2–4 | −1.0 ± 0.9 | 1.44 | 0.23 |
| 3–4 | 2.2 ± 1.1 | 4.22 | 0.04 |
| Density | | | |
| 1–4 | 1.3 ± 0.9 | 2.18 | 0.14 |
| 2–4 | −0.40 ± 0.6 | 0.39 | 0.53 |
| 3–4 | −2.3 ± 1.0 | 5.00 | 0.025 |
| Sex ratio | | | |
| 1–4 | 0.6 ± 0.7 | 0.65 | 0.42 |
| 2–4 | −1.3 ± 0.8 | 2.82 | 0.093 |
| 3–4 | 1.6 ± 0.7 | 5.24 | 0.022 |

[a]Only those parameters that had significant effects overall (Table I)
were included. The dependent variable was whether a male–male pair
was found in a given sample. There were five time periods (0700, 1000,
1300, 1600, 1900 h); other parameters were divided into quartiles due
to nonlinearity of effects.
[b]Note that these comparisons are necessarily relative and must use one
of the levels as a benchmark; therefore, the number of comparisons is
one less than the number of levels. The first number refers to the level
under consideration, the second to the comparison level.
[c]Negative values of parameters refer to a higher likelihood of finding a
male–male pair than in the comparison group; positive values refer to
a lower likelihood.

smaller than the bottom (top mean $= 5.61 \pm 0.081$ mm, bottom mean $= 5.88 \pm$
0.049 mm, mean difference [top − bottom] $= -0.26 \pm 0.085$ mm, paired
$t$ test, $t = 3.08$, $df = 22$, $P = 0.005$). In 2001, we simultaneously captured
unpaired males and heterosexually paired males, in order to compare the
sizes of the homosexual males to these males. The top male tended to be
smaller than both heterosexually paired and unpaired males, and the bottom
male tended to be larger than heterosexually paired and unpaired males,
but the difference was not significant for either top or bottom males (male–
male pairs, top $= 5.62 \pm 0.12$ mm, bottom $= 5.83 \pm 0.063$ mm, $N = 14$;
heterosexually paired males $= 5.69 \pm 0.029$ mm, $N = 100$; unpaired $= 5.68 \pm$
0.039 mm, $N = 62$; ANOVA for top vs. unpaired and heterosexual males,
$F_{2,173} = 0.370$, $P = 0.69$; ANOVA for bottom vs. unpaired and heterosexual
males, $F_{2,173} = 1.45$, $P = 0.24$).

## Laboratory Experiments: Density, Sex Ratio, and Temperature

In the laboratory, males readily paired with other males in a manner very similar to that of males pairing with females. That is, one male approaches another male, briefly runs its antennae along the dorsal surface of the male's body, and then mounts him. Males often extended their aedeagus toward the tip of the abdomen of the male they mounted but actual insertion was not observed in this study.

The results of our laboratory experiments indicated that density, sex ratio, and temperature have independent effects on male–male pairing. For density, the number of male–male pairs increased with the density of males ($20$—$1.08 \pm 0.19$ pairs, $30$—$1.6 \pm 0.27$ pairs; $40$—$4.6 \pm 0.30$ pairs; $N = 25$ for each density; Kruskal–Wallis nonparametric ANOVA, $H_{2,71} = 42.9$, $P < 0.0001$). The proportion of males in male–male pairs also increased with density ($20$—$0.11 \pm 0.019$, $30$—$0.11 \pm 0.018$, $40$—$0.23 \pm 0.015$; Kruskal–Wallis, $H_{2,71} = 24.8$, $P < 0.0001$), with the increase at the 40-male density being primarily responsible for the difference among groups.

For two of the three density treatments, the size of the individual was related to his position (top or bottom) within a male–male pair and his likelihood of being in a pair. For the 40-male treatment, in 79 of 114 pairs (69%), the top male was smaller than the bottom male (mean difference $= -0.22 \pm 0.042$ mm; paired $t$ test $= 5.25$, $P < 0.0001$). To determine whether the top and bottom males differed from the unpaired males, we averaged the sizes of the top, bottom, and single males within each trial and compared the averages within a trial (Fig. 2). In 22 of 25 trials, the top males were smaller than the unpaired males (paired $t$ test $= 4.1$, df $= 24$, $P = 0.0004$), and in 19 of 25 trials, the bottom males were larger than the unpaired males (paired $t = 3.5$, df $= 24$, $P = 0.002$). For the 30-male treatment, the top male was only slightly smaller on average than the bottom male (in 24 of 40 pairs; mean difference $= -0.02 \pm 0.063$ mm; paired $t$ test, $t = 0.32$, df $= 39$, $P = 0.75$). In addition, no significant differences existed between the top and the bottom males vs. the unpaired males on average (Fig. 2; top vs. unpaired: paired $t$ test, $t = 1.18$, df $= 17$; $P = 0.25$; bottom vs. unpaired male: paired t test, $t = 0.54$, df $= 17$, $P = 0.60$). For the 20-male trials, the top male was generally smaller than the bottom male (in 17 of 27 pairs; mean difference $= -0.16 \pm 0.076$ mm; paired $t$ test, $t = 2.19$, df $= 26$, $P = 0.038$). Within a trial for the 20-male treatment, the top males tended to be smaller (in 10 of 19 trials), and the bottom males larger (12 of 19 trials), than the unpaired beetles; however, these trends were not significant (Fig. 2; top vs. unpaired—paired $t$ test, $t = 0.73$, df $= 18$, $P = 0.48$; bottom vs. unpaired male—paired $t$ test, $t = 1.37$, df $= 18$, $P = 0.19$).
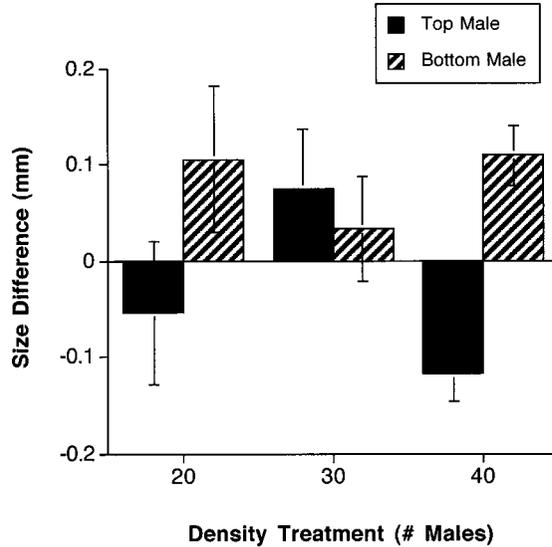
**Fig. 2.** Average size differences (±SE) within a trial for the top or bottom Japanese beetles of a homosexual pair as related to the males that were unpaired after 1 h for 20-male ($N = 19$ trials with homosexual pair), 30-male ($N = 18$ trials), and 40-male ($N = 25$ trials) density treatments.

In the sex ratio trials, the more male-biased the sex ratio, the more likely it was that a male–male pair occurred at some point in the trial (Fig. 3; $\chi^2 = 38.8$, df $= 4$, $P < 0.0001$) or was present at the end of 1 h ($\chi^2 = 18.3$, df $= 4$, $P = 0.001$) (note that these analyses use only the five treatments that included females). Similarly, the proportion of males that were homosexually paired increased with the sex ratio, whether we analyzed either the maximum number of male–male pairs (Spearman correlation, $r_S = 0.42$, $z = 3.79$, $P = 0.0002$) or the number of male–male pairs that existed at 1 h ($r_S = 0.45$, $z = 4.08$, $P < 0.0001$).

Temperature had a nonlinear affect on the number of male-male pairs. The most pairs were found at the lowest temperature ($23°C$: $1.97 \pm 0.22$ pairs, $N = 31$) and the highest temperature ($37°C$: $1.71 \pm 0.25$ pairs, $N = 34$), and the fewest pairs occurred at the middle temperature ($27°C$: $1.18 \pm 0.15$ pairs, $N = 33$ trials) (Kruskal–Wallis, $H_{2,94} = 6.36$, $P = 0.04$).

## DISCUSSION

In 3 years of sampling, we found that male Japanese beetles occasionally formed homosexual pairs in the field, with male–male pairs constituting
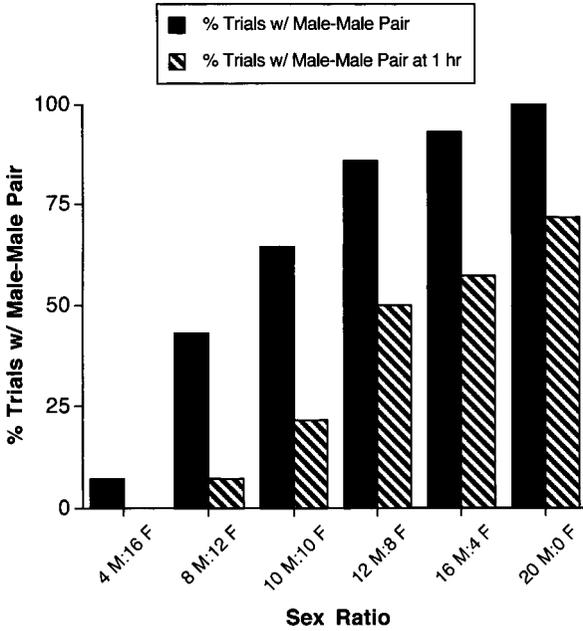
**Fig. 3.** Japanese beetle sex ratio (number of males:number of females) and the percentage of trials ($N = 14$) that had a male–male pair during any of the four sampling time periods (15, 30, 45, 60 min) and the percentage of trials that had a male–male pair during the last time period.

approximately 1–6% of the pairs. Japanese beetles also readily formed male–male pairs in the laboratory. To our knowledge, our study is one of the first to examine possible environmental effects on the frequency of homosexual behavior in insects. We found that the formation of these pairs was affected by time of day, male density, sex ratio, and temperature, but not in a linear manner. Homosexually paired males were not a random sample of males; top males were smaller than bottom males. A trend existed for unpaired males to be larger than top males and smaller than bottom males. Below, we first discuss the environmental factors that influenced this homosexual pairing, then suggest possible explanations for the size-related patterns and homosexual pair formation.

## Environmental and Social Factors

The highest frequency of male–male pairs was found in the afternoon. Four possible explanations could explain this pattern. First, the afternoon may be the time of day when the beetles are most sexually active. That is,

more male–male pairs form in the afternoon because that is when most heterosexual pairs are forming as well. However, the frequency of heterosexual pairs is opposite that of male–male pairs, with the highest frequency of heterosexual pairs occurring in the morning and the evening (Switzer *et al.*, 2001). Second, density may be higher or sex ratios may be more male-biased in the afternoon, which may lead to more male–male pairing. In our analyses, though, we statistically controlled for density and sex ratio differences, and previous analyses found no consistent differences in density or sex ratio at different times of day (Switzer *et al.*, 2001). Third, females, while present on the food plants, may have been either "hiding" from males or more actively rejecting males in the afternoon. Thus, the operational sex ratio of receptive females to receptive males may have changed, even though the absolute sex ratio did not. We have observed females pushing males away with their legs and crawling away from males that are attempting to mount them; these behaviors seem to qualify as rejection behavior (unpublished data). Finally, males may be spending less time guarding any particular female in the afternoon than they do in the morning and the evening. If males spend less time guarding females, more pairs may be forming. In the laboratory, male–male pairs last for a shorter time than heterosexual pairs (unpublished data). Therefore, even if pairs (homosexual or heterosexual) form at random, increased pairing may lead to a higher number of homosexual pairs in a given snapshot in time. These last two possibilities—female availability and male guarding time—remain to be tested.

In the laboratory experiments, sex ratio and density both were positively related to the frequency of male–male pairing. Increases in both led to increases not only in male–male pairing, but also in the proportion of males that were homosexually paired. Both sex ratio and density likely affect male–male pairing by being positively related to encounter frequency; increased density and increased sex ratio are going to increase the chance that a searching male encounters another male. If, for example, some underlying probability exists for a male to mount another male, increases in encounter frequency will increase the odds of a pair forming in a given amount of time (Serrano *et al.*, 2000).

Male–male pairs were more likely to be found at relatively low and high temperatures compared to more moderate temperatures. We suspect that this pattern may be due to the effect of temperature on pairing frequency and pair duration. Both flight activity (Fleming, 1972; Lacey *et al.*, 1994) and general activity (personal observations) are higher for Japanese beetles at higher temperatures; thus, more activity may increase mate searching behavior with more male–male pairing as a result. In addition, temperature may be negatively related to the duration of guarding, perhaps by being positively correlated with a physiological cost such as dehydration or energy expenditure

(Oertli and Oertli, 1990). Consequently, pairs at lower temperatures may last longer, and thus be more likely to be recorded, than those at higher temperatures. Our hypothesis is that the balance of these two effects—increased pair formation but decreased pair duration with temperature—leads to the nonlinear relationship between male–male pairing and temperature.

Overall, several environmental and social factors had a definite impact on the likelihood of male–male pairing. However, field and laboratory results did not completely correspond; although the same factors were important in both field and laboratory, the direction of the effects differed in some cases. Undoubtedly, the field situation is more complicated than our controlled laboratory conditions; in particular, light levels and availability of males and females certainly differed. Additionally, for most factors more than one possible mechanism could have caused the pattern we observed and these potential mechanisms remain to be investigated. Specifically, identifying the factors affecting the frequency of pair formation and the factors affecting the duration of pairs will help us understand how environmental and social conditions cause changes in apparent homosexual behavior.

## Possible Function of Male–Male Pairs and Potential Mechanisms for Pair Formation

Harari *et al.* (2000) outlined four possible hypotheses for the function of homosexual behavior: female mimicry by inferior males, dominance behavior, sperm transfer from male to male (i.e., with the result of a male passing another male's sperm to a female upon copulation), and mistake in identifying females. The first three of these hypotheses suggest that male–male pairing is adaptive behavior that has direct benefits for one of the individuals in the pair, while no immediate benefits occur for the mistake hypothesis. Of these four hypotheses, the available evidence for Japanese beetles strongly supports the mistake hypothesis. Male–male pairs were relatively rare in the field, which makes the adaptive explanations less likely. In addition, larger males seem to have a competitive advantage in Japanese beetles (unpublished data), and in this study we found that the males that are mounting other males tended to be relatively small. Furthermore, although copulations are attempted in homosexual pairs, successful "copulation" in homosexual pairs is extremely rare (much less than 1% of attempted copulations in the laboratory [unpublished data]). Given this information, the larger, bottom male was unlikely to be inferior and thus mimicking a female (female mimicry hypothesis), the smaller, top male was unlikely to be dominant to the bottom male (dominance hypothesis), and no sperm would be transferred from the small male to the large male (sperm transfer hypothesis)

(Harari *et al.*, 2000). Potentially, large males could be attracting small males in some manner (i.e., large males "intentionally" mimicking females), but likely costs of this behavior in this system (e.g., increased competition for mates) make it unlikely that this behavior would yield a net benefit to the large male. In support of the mistake hypothesis, females on average are larger than males but there is considerable overlap in the size distributions (Van Timmeren *et al.*, 2000; Switzer *et al.*, 2001) and the sex pheromone, although released by virgin females, is not released by nonvirgin females (i.e., the females found on the food plants) (Ladd, 1970). Thus, size may be one of the only initial cues available to males for sex discrimination (see also Harari *et al.*, 2000). Therefore, although this study was not designed to test functional hypotheses for homosexual behavior in Japanese beetles, our data and other observations allow us to reject the female mimicry, dominance, and sperm transfer hypotheses and support the mistaken identity hypothesis.

The general pattern that we observed was that the top male of a homosexual pair tended to be smaller than the bottom male. Harari *et al.* (2000) observed a similar size pattern in homosexual pairs of the beetle *Diaprepes abbreviatus*. This size pattern suggests a few, nonexclusive mechanisms that might be responsible for the formation of male-male pairs. First, males may actively be choosing other males because they mistake them for females (Harari *et al.*, 2000). Larger males are more likely to be mistaken for females, either as a direct result of using size as a cue for sexual identity or because large males are more likely to mate and consequently have some female sexual pheromone on them. Either way, the top male would tend to be smaller, even if all males were equally likely to homosexually pair. Second, relatively small males may be less discriminating or selective than relatively large males (e.g., Foote, 1988; Dunn *et al.*, 1999), which may lead to more mistakes by smaller males. Third, sexual confusion may result during competition for females. Males do attempt to take over females from paired males in the field (Fleming, 1972; Potter and Held, 2002); we have observed (unpublished data) that occasionally during these takeover attempts, males seem to get "confused" as to which of the individuals is the female and which is the male, and homosexual pairs result (with the female often leaving or remaining solitary). However, while this process may explain the formation of pairs in general, it does not explain the size pattern unless this confusion is more likely with a relatively large male guarding the female or unless small males are more likely to attempt takeovers of existing pairs. Given our current understanding of the system, these potential mechanisms are all plausible. Additionally, these mechanisms are all from the perspective of the small, mounting male and, therefore, do not account for the behavior (e.g. resistance) of the large, mounted male. Further testing of these mechanisms will require detailed observations of the formation and maintenance of homosexual pairs.

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