

Role of contests in the scramble competition mating system of a leaf beetle

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Running Head: Mating behavior in a montane leaf beetle

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ABSTRACT

Male competition for mates can occur through contests or a scramble to locate females. We examined the significance of contests for mates in the leaf beetle *Chrysomela aeneicollis*, which experiences a short breeding season. During peak mating season, 18-52% of beetles are found in male-female pairs, and nearly half of these are copulating. Sex ratios do not differ from parity, females are larger than males, and positive size-assortative mating occurs. Males fight (2-4% of beetles) over access to females, and disruption of mating usually follows these contests. In the laboratory, we compared mating and fighting frequencies for males found in mating pairs (field-paired) and single males placed into an arena with a field-paired female. Mating pairs were switched in half of arenas (new male-female pairs) and maintained in the other half. For 2 days, each male was free to move about and fight; thereafter males were tethered to prevent contests. Mating frequencies were significantly greater for field-paired than single males in both situations. Male size was not related to mating frequency; however, large females received more matings than small ones. These data suggest that males fight for high quality females, but otherwise search for as many matings as possible.

Keywords: scramble competition, mating behavior, Chrysomelidae, mating system, contest competition

INTRODUCTION

Insects display a wide range of mating systems and vary in degree of sexual dimorphism, sex determination factors, level of inbreeding, and influence of territoriality and contests on mating success (Alcock, 1998). In most insects, males compete against each other for access to potential mates by interfering with other males that are mating (interference competition), or by outpacing other males in the search for suitable mates (scramble competition). Characterizing the mating system of a species provides insights into opportunities for sexual selection to act on body size and behavioral characters (Thornhill and Alcock, 1983). When interference competition is important, one would expect a positive relationship between male size and mating success, frequent contests among males for access to females, resource or territorial defense by males, and a positive correlation between female and male size in mating pairs (positive assortative mating). Scramble mating systems are typically characterized by low sexual size dimorphism, no large-male advantage in mating, little or no assortative mating, and few contests among males for mates (Thornhill and Alcock, 1983). In nature, mating systems often fail to fall neatly into one or another category. Contests among males may occur when scramble competition is intense, and scramble competition may play a role in a system characterized by interference (Parker, 2000). Thus, selective pressures on male and female traits will depend on the relative importance of scrambling versus interference (Blanckenhorn *et al.*, 1995; Blanckenhorn, 2000; Kraushaar and Blanckenhorn, 2002, Bertin and Cezilly, 2003, Eberle and Kappeler, 2004, Spitzer *et al.*, 2004). Few studies have investigated the importance of contests in a scramble competition insect mating system (Bean and Cook, 2001).

We studied the mating system of the leaf beetle *Chrysomela aeneicollis* in the Eastern Sierra Nevada mountains in California, USA. Study populations occur on the edge of this species

range, where beetles experience a short breeding season under stressful climatic conditions (Dahlhoff and Rank, 2000; Rank and Dahlhoff, 2002). Adults emerge from diapause after snowmelt and actively feed and mate on willows throughout the day when temperatures are warm (Rank, 1992; Dahlhoff and Rank, 2000). After emergence, females and males mate with multiple partners in large aggregations on host plants for 2-4 weeks. Females tend to be larger than males and males possess no exaggerated ornaments or structures (Brown, 1956). Contests among males occur in nature. Nevertheless, there is no evidence that males monopolize territory or resources, as beetles are scattered throughout host plant foliage. There is also no evidence for courtship behavior and no obvious signs of rejection of males by females.

The existence of contests between males suggests that interference competition operates, yet the absence of sexual size dimorphism in favor of males and the lack of resource defense suggests that scramble competition for mates occurs. To characterize the mating system of *C. aeneicollis*, we assessed the frequency of common behaviors in natural populations during the breeding season, and determined whether the sexes differ with respect to frequency of each behavior. We also determined if females and males differ with respect to morphological characteristics related to body size, and if there is evidence for size-assortative mating. We then determined the outcomes of contests among males in the field and laboratory, and assessed how body size, mating status in the field, and previous experience with a mating partner relate to mating and fighting frequency in males.

METHODS

Field observations

We observed single beetles and beetles in mating pairs in a population in Big Pine Creek (Falls Site, 37° 06'N, 118° 29'W, elevation 2940 m) and two populations in Bishop Creek (South Lake, 37° 10'N, 118° 33'W, 3000 m; Bluff Lake, 37° 11'N, 118° 33'W, 3200 m). Behaviors observed included sitting, walking, feeding, or fighting over access to a mate (males only). Beetles were counted as single or paired (*in copula* or with the male on the female's back). One observer scanned host foliage from 1 meter distance for 30-140 minutes at mid-day while beetles were active. Each observation period was ended once adequate sample size was obtained. Counts were performed 2-3 weeks after snow melt (16-19 July, 1998) when adult beetles were abundant. On the morning of 19 July 1998, we collected 188 beetles at Bluff Lake, found singly or in mating pairs, which were sitting, walking, or feeding at field collection. We subsequently determined the sex of these beetles and calculated sex ratios for them and for 466 additional beetles collected at Bluff Lake during 13-17 June 2000. We also conducted a day of observations on 2 July 2005 near South Lake in Bishop Creek, to confirm observations made in prior field seasons.

To assess the outcome of contests among males in nature, we observed behavioral interactions among individuals at Bluff Lake during the middle of the breeding season in July 1999. From mid-morning until late afternoon, we inspected host plants to locate two males fighting over a female. We selected trios where a male had attempted to dislodge a male from a mating pair for further observation. We designated the mating male the 'resident' and his opponent the 'intruding' male. Over two days, we located 37 contests and recorded observations until the contest ended.

Laboratory mating trials

On 5 July 1999, we collected male/female mating pairs and single males at Bluff Lake and brought them to the White Mountain Research Station (Bishop, CA) for laboratory experiments. We held beetles for 48 hours as mating pairs or singly prior to initiating trials. Before trials, we marked each male with a small spot of model paint and placed beetles in trios (2 males, one female) into mating arenas (14 cm Petri dishes). In half of the mating arenas, we placed the field-paired male with the same female that he had mated at field collection (field mating pair) and a randomly-selected single male. In the remaining arenas, we placed the field-paired male with a different field-paired female and a single male (switched mating pair). Beetles were kept at 22°C during the day and at 5°C at night.

During the first two days of laboratory mating trials, all beetles moved freely within the arena. Males were able to interact with each other and the female at will. At the end of the two days, we tethered each male to a different side of the mating arena. A tether consisted of a 6 cm long thread taped to the side of the dish and attached with a 1 mm wax ball to each male's elytron. Males were no longer able to interact physically, and females could avoid physical contact with both tethered males. After tethering, we observed each trio for three days. The tethered trial was longer than the contest trial because mating frequencies declined after tethering.

We observed all mating arenas twice every hour for 12 hours during the day and recorded whether each beetle was mating, feeding, walking, or sitting (females and males), or fighting or mate-guarding (males only). For each time point and behavior, we recorded a '1' if the animal was engaged in that behavior and a 'zero' if it was not. We took averages of all time points to estimate proportion of time spent in each behavior. During the male contest trial, we recorded

timing and frequency of contests among males. We also analyzed the relationship between number of contests and proportion of observations where mating occurred. Most contests occurred while one male was in a mating pair. We identified him as resident male and the other as intruding male.

Body size measures

When trials were completed, all beetles were frozen at -80 °C until measurements were made. We measured thorax width, elytra length and front femur length of each beetle to the nearest 0.002 mm twice using orthogonal Acu-rite micrometers on a Leica MZ12 microscope. To quantify thorax width, we measured pronotum width at attachment to the mesothorax. We measured each elytron between posterior tip and anterior margin and each front femur at the longest point. To estimate body size, we conducted principal components analysis of these three variables on all beetles.

Data analysis

We used one-way ANOVA and Tukey's Honestly Significantly Different tests to compare values for each body size measure and PC1 in females, single males and paired males. We also determined whether PC1 values of females were related to those of their male mates at field collection. Statistical analyses were conducted in JMP IN (5.1, SAS Institute). We analyzed laboratory mating trial results using two-way ANOVA, with proportion of observations of each behavior for each male as dependent variables. Grouping factors included 'pair type' (field mating pair or switched pair), 'mating status' at time of collection (field-paired or single male), the interaction between pair type and mating status, and 'dish' (each mating arena). Dish was considered a random effect, analogous to 'block' in a randomized blocks design (Sokal and Rohlf, 1981). Inclusion of 'dish' in the ANOVA removed corresponding degrees of freedom

from the error term, and allowed for statistical tests based on number of dishes rather than number of insects. With this approach, multiple experimental factors and covariates can be evaluated in a single statistical model (Quinn and Keough, 2002).

During laboratory mating trials, some behaviors were observed relatively rarely, yielding a high proportion of zeroes. When the proportion of zeroes exceeded 12% (e.g. mating in the tethered trial), residuals in preliminary ANOVAs were not normally distributed. We transformed this variable into a dichotomous response variable (0 = mating never observed, and 1 = mating observed at least once for that male) and analyzed data with a generalized linear model and likelihood-ratio tests (Hardy and Field, 1998), using the statistical model described above. The statistical power of generalized linear models is lower than that of parametric statistical tests (Quinn and Keough, 2002); thus, we used this approach only when violation of ANOVA assumptions required it.

We determined whether male body size was related to mating frequency in the contest trial using analysis of covariance (ANCOVA). Mated and single males were analyzed separately, with 'pair type' as grouping factor, PC1 as covariate, and the interaction between PC1 and 'pair type' to test for heterogeneity among slopes (Quinn and Keough, 2002). To determine whether male size was related to male mating frequency in the tethered trial, we used a generalized linear model with effects described above, and presence or absence of mating as dependent variable. To relate female body size to her mating frequency, we calculated the sum of each female's mating frequencies with both males during the contest trial, and conducted ANCOVA with 'pair type' as grouping factor. The interaction between 'pair type' and PC1 tested whether the relationship between size and mating frequency depended on whether the female was placed with her field mating partner or a new male.

RESULTS

Field observations

We found that when a male encounters a female during the breeding season, he often attempts to mount her, and if he does, he usually attempts copulation immediately. During copulation, males sit, or may drum antennae or front femora, and females sit, walk, or feed. Many females are obviously gravid, and they occasionally try to oviposit while the male is attempting to mate. We observed no struggles between females and males. Some females walk rapidly away from a male that is attempting to mate, or from two males that are fighting to mate with her, which might indicate rejection. On a typical afternoon (2 July 2005), 240 of 475 beetles (51%) were found in mating pairs. For the majority (55%) of mating pairs, beetles were not in copula, but the male was sitting on the female with his abdomen partially covering hers. We refer to this behavior as ‘mate-guarding,’ whereas beetles in copula are defined as ‘mating.’ During the 2 July observation, eight contests between males were observed. Six contests involved two males and one female, and two contests involved three males fighting over one female. Thus, 4% of beetles observed were involved in contests.

Frequency of beetle behavior was quantified for 1998 field populations (Table 1). Beetles were usually found sitting, feeding, or walking, and the proportion of individuals mating tended to increase between morning and afternoon (Table 1). Mating pair frequencies were lower at Bluff Lake and South Lake in Bishop Creek than at Falls Site in Big Pine Creek, probably because snowmelt occurred later at Falls Site that year. Contests between males occurred throughout the day, but the frequency of beetles observed fighting was low (< 2%; Table 1).

Most of 188 beetles collected in Bluff Lake in 1998 were unpaired (66%) when collected. Among single beetles, most females (67%) and males (59%) were sitting at collection. The next

most common behavior found in males was walking (37%). Only a few (4%) males were feeding at collection, whereas more females were feeding (19%) than walking (14%). These differences in behavior between single females and males were highly significant ($G = 13.8$, d.f. = 2, $P = 0.001$). All mating males were sitting on the female, and most mating females (81%) were sitting on a host leaf or shoot. The remainder of mating females were feeding or walking. The observed sex ratios in 1998 (44% female) and 2000 (466 beetles, 54% female) were not significantly different from parity (goodness of fit test; 1998: $G = 2.58$, d.f. = 1, $P > 0.1$; 2000: $G = 3.4$, d.f. = 1, $P = 0.064$).

Natural contests were recorded at Bluff Lake during the 1999 field season. In 12 of 37 contests observed, resident males were copulating when an intruding male approached, and in the others, he was mate guarding. Contests lasted 2-88 min (mean \pm SD = 7.9 ± 14.3 min). During contests, males pushed each other or grasped each other's legs, antennae or body with their mandibles. Intruding males often extruded genitalia while trying to dislodge the resident male. The female often continued walking while males fought, and if she became separated from the fighting males, she usually (65% of contests) did not pair with either male afterwards. Males continued their contest for several seconds after the female had walked away, and appeared to search for the female after the contest was over. If neither male located the female immediately after the contest, the males walked away from each other. The intruding male was observed mating with the female in three of 13 contests where mating was observed at the contest's end. We observed no injuries after contests, but males with missing legs are occasionally encountered during the breeding season (N. Rank, personal observations).

Body size and mating in nature

Females were significantly larger than males in elytra length ($F_{2,94} = 213.2, P < 0.0001$), thorax width ($F_{2,95} = 63.3, P < 0.0001$) and femur length ($F_{2,94} = 29.9, P < 0.0001$, Table 2). Field-paired males did not differ from single males with respect to any variable (Table 2). The first principal component (PC1) explained 88% of the variation in the three morphological variables and was positively correlated to each one ($r = 0.57-0.58$). PC1 scores differed between females and males ($F_{2,93} = 88.0, P < 0.0001$), and PC1 values for females were positively related to those of their mates at field collection (Fig. 1).

Male contest trial

Mating occurred throughout each day of the contest trial and was observed at least once at every time point. The proportion of individuals mating increased during the first two hours of observation, and remained fairly constant until late afternoon (declining during the last three hours of observation on the first day). Mating occurred within the first three hours of observation for 80% of dishes (mean time to first mating = 2.8 ± 0.9 hr). Mating and mate-guarding occurred in every dish, but mate-guarding was observed less often than mating (Wilcoxon signed-ranks test: $N = 33, T = -143, P < 0.005$). The first observation of mate-guarding usually occurred after the first mating (75% of dishes). Most matings (79%) were only observed at one time point, but 16% were recorded over two consecutive observations and the remainder over three or four observations. Contests among males occurred in 75% of dishes, but they usually occurred after the first mating (mean time to first contest = 7.7 ± 1.9 hr).

During the contest trial, field-paired males mated more often than single males (Fig. 2A, $F_{1,31} = 17.4, P < 0.001$). This difference tended to be greater in switched pairs (male with new female) than in field-mating pairs (interaction term; $F_{1,31} = 3.2, P = 0.09$). In two thirds of

dishes, the field-paired male mated before the single male (goodness of fit test $G = 3.74$, d.f. = 1, $P = 0.053$). Similar patterns were observed for mate guarding (Fig. 2B). Field-paired and single males did not differ with respect to frequency of fighting, feeding or walking, and frequencies of these behaviors did not differ between field and switched mating pairs (Table 3, $P > 0.2$ for all comparisons). Single males spent more time sitting than field-paired males (Table 3, $F_{1,31} = 14.0$, $P < 0.001$), and this difference was greater in switched than in field pairs (interaction term; $F_{1,31} = 5.0$, $P = 0.03$).

A total of 97 contests among males were observed. Number of contests was positively related to total number of matings (Fig. 3A), but did not depend on field/switched pair type ($F_{1,31} = 0.43$, $P > 0.5$). The proportion of matings by the single male increased with contest number (Fig 3B), while the proportion of matings by the field-paired male decreased (data not shown; proportions sum to one). In 84% of dishes where contests occurred, both males mated with the female afterwards, and in the other four dishes, where there were few (1-2) contests, only the field-paired male mated after the contest. For 14 contests, mating was recorded at the next time point. In half of these observations, the female was paired with the male that had been mating with her before the contest (resident), and in other half, she was paired with the male that had initiated the contest (intruder). Four successful intruders were single males, and three were field-paired males. Of seven subsequent matings with resident males, two were with single males (one involved in two contests) and four were with field-paired males.

Tethered trial

During the tethered trial, mating only took place when a female approached a tethered male and remained near him long enough for copulation to occur. As observed during the contest trial, the proportion of individuals mating increased during the first three hours of observation. It

decreased towards the middle of each day, peaked again late in the afternoon, and declined at the end of the day. Mating and mate-guarding frequencies declined between contest and tethered trials (Wilcoxon signed-ranks tests, $N = 32$; mating $T = 262$, $P < 0.0001$; mate-guarding $T = 276.5$, $P < 0.0001$). No mating or mate-guarding was observed for 13 of 33 dishes, and mean time to first mating (8.3 ± 2.1 hr) was greater during the tethered trial. Most matings (89%) were observed at only one time point. On a few occasions, while a female was mating with one male, we observed the other male straining at the end of his tether, attempting to approach the mating pair. We observed no displays by males towards each other or the female.

During the tethered trial, field-paired males were observed mating more often than single males (Fig. 2C, generalized linear model, $G = 17.4$, $P = 0.028$). In 78% of dishes where mating occurred, the female mated with the field-paired male before the single male (goodness of fit test $G = 7.8$, d.f. = 1, $P = 0.005$). Similar patterns were observed for mate guarding (Fig. 2D). As in the contest trial, males did not differ in proportion of time spent walking, sitting, or feeding, and there was no effect of pair type on these behaviors (Table 3, $P > 0.15$ for all comparisons).

Body size versus mating frequency

Male body size (PC1) was not related to mating frequency for field-paired males (contest trial: ANCOVA $F_{1,28} = 0.48$, $P > 0.50$; tethered trial: $G = 2.47$, d.f. = 1, $P = 0.11$) or single males (contest: ANCOVA $F_{1,30} = 1.0$, $P > 0.30$; tethered: $G = 0.44$, d.f. = 1, $P > 0.50$). However, the relationship between female body size and mating frequency depended on pair type during the contest trial (Fig. 4, ANCOVA interaction $F_{1,28} = 8.13$, $P = 0.008$). Female mating frequency was positively related to her body size in field mating pairs, but not in switched pairs.

DISCUSSION

Many species of insects and some vertebrates reproduce in dense aggregations during a brief period, where the most successful male strategy is to outrace other males to locate and copulate with females before other males mate with them (Thornhill and Alcock, 1983). In these ‘scramble competition’ mating systems, males focus on mate location rather than defending territories or engaging in prolonged contests over access to females (Alcock, 1998). Our observations of *C. aeneicollis* are consistent with this pattern, except that aggressive interactions among males and contests for mates play a role in its mating behavior. Other studies have also found contests among males in mating systems that are best described as scrambles (Dickinson, 1992; Goldsmith *et al.*, 1996).

Contests among males play a significant role in the mating behavior of *C. aeneicollis*. Although single males received fewer matings than field-paired males in the contest trial, single males that engaged in contests increased their mating frequencies substantially. On the other hand, contests among males did not result in monopolization of females, as both males usually mated before and after a contest. In the field and laboratory, most contests did not conclude with mating. When males encounter a female with another male that is already mating or mate-guarding, they may attempt to dislodge the resident male, but if they do not succeed, they search for another female. Thus, in this species, males appear to adopt the tactic of engaging in contests while searching for mates.

Although large-male mating advantage is common among many insects, other studies have also found no size difference between mating and single males (Dickinson, 1992; Brown, 1993; Goldsmith *et al.*, 1996; Jaastad, 1998; Peckarsky *et al.*, 2002). A lack of a large-male mating advantage is frequently observed in scramble mating systems where females are larger than

males. Large body size can be disadvantageous for males that are scrambling or climbing to find mates (Blanckenhorn *et al.*, 1995; Blanckenhorn and Viele, 1999; Dunn *et al.*, 1999; Blanckenhorn, 2000; Stoks, 2000; Moya-Larano *et al.*, 2002). Large body size is not always advantageous in contest competition either (Dickinson, 1992, Blanckenhorn *et al.*, 1995, Goldsmith *et al.*, 1996). For example, many butterflies where males defend territories or resources, large male body size is not associated with victory in contests or with mating success (Hernandez and Benson, 1998; Kemp and Wiklund, 2001).

We found size-assortative mating in the field, but there was no difference in body sizes of mating versus single males in nature. We also observed no large-male advantage in the laboratory. Several hypotheses may explain the presence of size assortative mating in nature without a large-male mating advantage. One possibility is that mating is most successful if female and male size is relatively closely matched (Brown, 1993). Since males ride on females while mating and mate-guarding, females may reject males that are excessively large. Another possibility is that both sexes prefer larger mating partners under field conditions, leaving small individuals to mate with other small individuals (Snead and Alcock, 1985; Brown, 1990; Capone, 1995). A third possibility is that body size relates to competitive ability in nature even if it did not in the laboratory.

The positive relationship between number of male contests and number of matings in the laboratory suggests that males were more motivated to compete for and mate with some females than others. Fecundity depends on female size in *C. aeneicollis* (Bruce, 2005) and other insects (Thornhill and Alcock, 1983; Baur and Rank, 1996; Sokolovska *et al.*, 2000; Kraushaar and Blanckenhorn, 2002). Males often prefer to mate with larger, more fecund females over smaller ones (Lawrence, 1986; McLain, 1988; Brown, 1990; Pitafi *et al.*, 1990; Capone, 1995; Bateman,

1998; delCastillo *et al.*, 1999; Goulson *et al.*, 1999). In other organisms, male investment in contests depends on perceived potential return for contest behavior (Murai *et al.*, 2002). In the present study, large females received more matings than small ones in field pairs, suggesting that males were more motivated to compete for more fecund females.

The effect of pair type (field versus switched) on the relationship between female body size and number of matings suggests that male mating behavior also depends on whether the female was new to both males. Repeated copulation with the same female often provides diminishing reproductive returns (Dickinson, 1986; Reinhold *et al.*, 2002; Lewis, 2004). Arnaud and Haubruge (1999) found that males preferred to mate with novel females over females that they had previously mated. The effect of pair type on the relationship between female size and mating frequency in the present study was likely influenced by differences in the field-mating males' investment in mating activity, and probably reflects the tendency for these males to allocate more reproductive effort to novel females.

Tethering males eliminated the possibility of physical contests among males for mates. After tethering, frequencies of mating-associated behaviors declined dramatically, while frequencies of other behaviors increased for both sexes. Females often appeared to avoid mating when males could not pursue them, as evidenced by the steep decline in mating frequency. The large decline of mate guarding during the tethered trial suggests that females did not remain close enough to the males for them to guard after tethering. These data suggest that females chose to mate with the same males that had mated with them during the contest trial. On the other hand, we cannot rule out the possibility that tethered males competed by sending chemical or visual signals to females or each other during the trial (Lewis and Austad, 1994; Birkinshaw and Smith, 2001).

The present study reveals evidence for male competition and mate preference in a mating

system that has many features of scramble competition. By manipulating mating history in a comparison between males of different field mating status, we detected differences among males in mating frequency and in tendency to engage in contests for a mate. Our study revealed a high correspondence between field and laboratory mating behaviors, and suggests a role of female body size and novelty in mate choice. These results support Wiley and Poston's (1996) view that choice and competition influence each other to mold the context within which sexual selection operates.

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Table 1. Proportion of beetles observed engaging in four behaviors in nature. Data collected at Falls Site (Big Pine Creek) on July 15 1998 and two sites in Bishop Creek (High Stream Crossing, South Lake) on July 19 1998.

	Falls Site			Bluff Lake		South Lake	
Time of day	6:50 a.m.	9:50 a.m.	3:30 p.m.	8:30 a.m.	1:45 p.m.	9:30 a.m.	2:00 p.m.
Sample size	(<i>N</i> = 308)	(<i>N</i> = 628)	(<i>N</i> = 362)	(<i>N</i> = 226)	(<i>N</i> = 278)	(<i>N</i> = 349)	(<i>N</i> = 372)
Percent paired	45.5%	49.4%	51.4%	28.3%	33.1%	18.3%	28.5%
Beetles in mating pairs							
Fight	1.0%	0.8%	1.7%	0%	0%	0%	0.3%
Walk	0.6%	3.2%	1.7%	0%	0.7%	0.6%	1.1%
Feed	3.2%	2.9%	0%	2.7%	2.9%	0.6%	1.6%
Sit	40.6%	42.5%	48.1%	25.7%	29.5%	17.2%	25.5%
Single beetles							
Fight	1.0%	0.8%	1.7%	0.0%	0.0%	0.0%	0.3%
Walk	4.9%	14.3%	11.3%	3.5%	8.6%	8.9%	12.6%
Feed	3.2%	7.0%	11.6%	4.9%	6.1%	10.6%	13.4%
Sit	45.5%	28.5%	24.0%	63.3%	52.2%	62.2%	45.2%

Table 2. Body sizes of beetles collected in mating pairs and single males. Superscripts indicate different means (Tukey Kramer HSD Multiple Comparisons Tests).

Character	Field-Paired Female			Field-Paired Male			Single Male		
	<i>N</i>	Mean	± S.E.	<i>N</i>	Mean	± S.E.	<i>N</i>	Mean	± S.E.
Elytron (mm)	32	5.41	± 0.03 ^a	32	4.52	± 0.03 ^b	33	4.59	± 0.04 ^b
Femur (mm)	33	1.36	± 0.01 ^a	31	1.28	± 0.01 ^b	33	1.29	± 0.01 ^b
Thorax (mm)	33	2.58	± 0.02 ^a	32	2.32	± 0.02 ^b	33	2.34	± 0.02 ^b

Table 3. Proportion of observations spent in behaviors (other than mating or mate-guarding) for field-paired versus single males, and for field-paired females, in laboratory trials. Field pairs included the same female and male that had been mating in the field ($n = 17$). In switched pairs, the field-paired male was placed with a different female ($n = 16$).

	Male behavior				Female Behavior	
	<u>Field pair</u>		<u>Switched pair</u>		<u>Field Pair</u>	<u>Switched Pair</u>
Contest trial						
	Paired male	Single male	Paired male	Single male		
Fight	0.06	0.06	0.05	0.05	----	----
Walk	0.19	0.24	0.18	0.19	0.15	0.15
Feed	0.09	0.08	0.07	0.09	0.07	0.06
Sit	0.40	0.46	0.33	0.57	0.34	0.30
Tethered trial						
	Paired male	Single male	Paired male	Single male		
Walk	0.24	0.24	0.26	0.20	0.29	0.25
Feed	0.12	0.11	0.12	0.13	0.13	0.16
Sit	0.60	0.62	0.58	0.65	0.53	0.53

FIGURE CAPTIONS

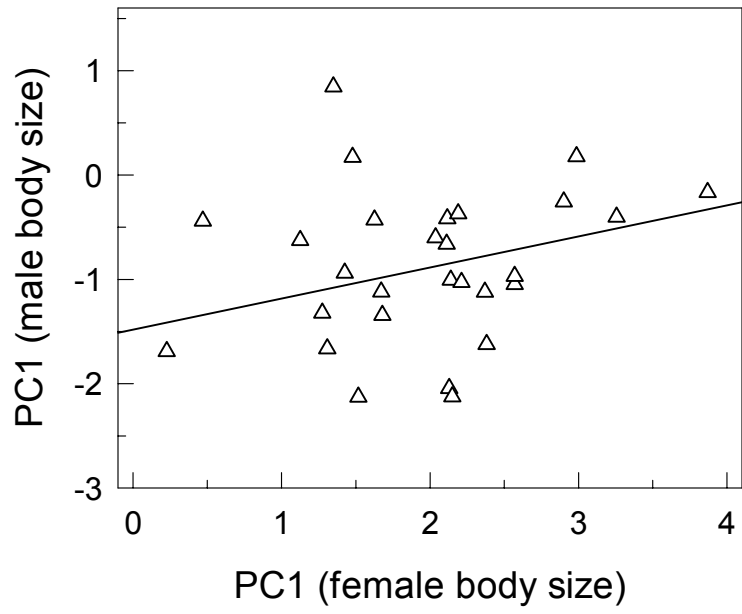
Fig. 1. Relationship between female and male body size for 29 pairs mating at field collection ($Y = 0.336X - 1.54$, $R^2 = 0.17$, $F_{1,27} = 5.62$, $P = 0.025$). The first principal component (PC1) was based on three morphological variables (thorax width, elytra length, and front femur length) measured for 99 beetles and explained 88% of the variation in these features.

Fig. 2. Mating (A, C) and mate guarding (B, D) during contest (A, B) and tethered (C, D) trials. Data shown are least squares means (\pm SE) of proportion of each male behavior for field-paired males (striped bars) or single males (solid bars) observed in $n = 17$ arenas where female was paired with her field mating partner (field pairs) and $n = 16$ arenas where female was paired with a new male (switched pairs). Mating or mate-guarded females were usually sitting (74% of observations), followed by walking (21%) and feeding (5%), during the contest trial. Other behaviors observed during contest and choice trials described in Table 3.

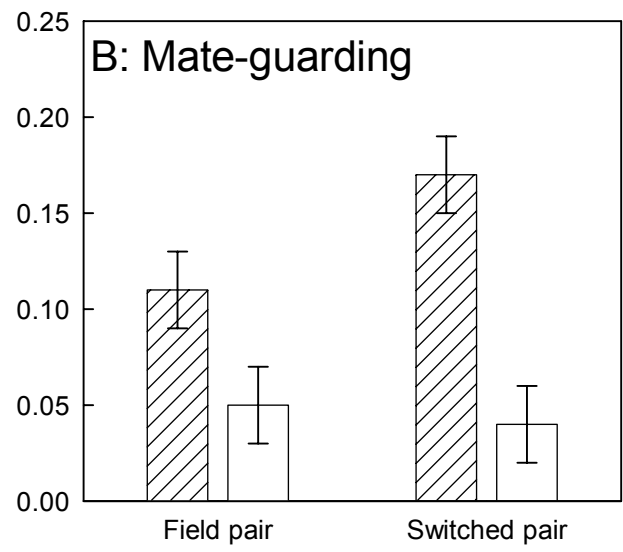
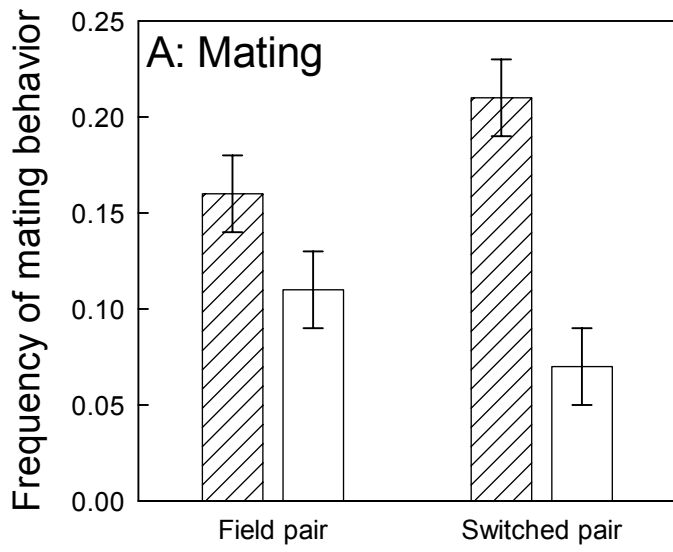
Fig 3. A. Relationship between number of male contests and number of matings received by females during contest trials ($Y = 0.63X + 8.6$, $R^2 = 0.21$, $F_{1,31} = 8.32$, $P = 0.007$). **B.**

Relationship between number of male contests and proportion of matings obtained by single males during contest trials ($Y = 0.028X + 0.21$, $R^2 = 0.13$, $F_{1,31} = 4.9$, $P = 0.034$). Solid circles represent field pairs, open circles switched pairs.

Fig. 4. Relationships between female body size and frequency of mating during contest trials. Solid circles indicate field mating pairs (solid line: best linear fit) and open circles indicate switched mating pairs (dashed line: best linear fit). Statistical analyses indicate that the relationship between female body size and mating frequency depended on pair type (interaction term in ANCOVA, *see Results text*).



Contest trial



Tethered trial

