Factors Influencing Mate Choice in *Euschistus conspersus* Uhler (Heteroptera: Pentatomidae)

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ABSTRACT The patterns of mating and possible factors influencing mate choice in the consperse stink bug, *Euschistus conspersus* Uhler, were studied in a series of laboratory experiments. Males were found to transfer a significant percentage of their body mass during the initial mating. Mating was also found to reduce male longevity by 37.8% but had no significant effect on female longevity or fecundity. There was no evidence of male or female choice based on weight of potential mating partners. There was assortative mating based on experience, however, with males mating preferentially with virgin females and females preferring mated males when given the choice. The implications of these findings within the mating dynamics of this aggregating species are discussed.

KEY WORDS *Euschistus conspersus*, reproductive behavior, mate choice, mating behavior, parental investment

The mating period of *Euschistus conspersus* Uhler (Heteroptera: Pentatomidae) in northcentral Washington occurs once each year, during spring through early summer. During this time, *E. conspersus* are often found in aggregations, typically consisting of 10–40 individuals, on host plants (Krupke et al. 2001), a response that is mediated, at least in part, by a male-produced aggregation pheromone component (Aldrich et al. 1991, Krupke et al. 2001). However, there is little published information detailing what actually occurs within these aggregations once they have formed. Mate choice, for example, has not been examined in this species.

To study mate choice in any species, the relative roles and contributions of the sexes must first be identified. Parental investment theory (Trivers 1972) predicts that females in species where males invest heavily in offspring fitness should compete for resources and/or for sexually active males and that the reverse is true in species where females invest more heavily. In heavy male investment situations, males may be expected to exhibit choosiness in mate selection. The first step in making predictive hypotheses about the roles of males and females in the *E. conspersus* mating system is to document how each sex might maximize its reproductive fitness. For example, in male insects of many species, one way of improving fitness is associated with the mass of material transferred to the female during mating. Material transfer

has been shown to play a role in the mating dynamics of stink bugs and a variety of other insect species. For example, virgin males of the red-shouldered stink bug, *Thyanta pallidovirens* Stal, have been shown to donate a large fraction ($\approx$17%) of their body mass to the female during the mating sequence (Wang and Millar 1997), whereas studies of the southern green stink bug, *Nezara viridula* L., revealed that large males mated more often (and with greater fertilization efficiency) than small males (McLain 1980). In many species of Orthoptera, males make a comparable sacrifice—about one fifth of their body weight—given as a spermatophore during mating (Gillette 1904). In bush-crickets, this spermatophore was found to be proportional to the size of the donor, and studies showed that females showed a preference for larger males (Gwynne 1984). In other insect species, masses of material have been shown to act as mating plugs that enhance mating success by a variety of mechanisms, including inducing behavioral or physiological changes in mated females (Wolfner 1997, Yi Shu and Gillott 1999), inhibiting or physically blocking further matings (Monnin and Peeters 1998, Polak et al. 1998), and preventing sperm from flowing out of the female reproductive tract (Woyciechowski et al. 1994).

The aforementioned factors may constitute a heavy male parental investment, perhaps leading male *E. conspersus* to exhibit mate choice. To determine whether male mate choice does occur in *E. conspersus*, we studied one aspect of male parental investment by quantifying the amount of weight change during mating and the effects of mating on male and female longevity. We also describe a series of experiments

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examining the possible roles of size and experience in determining male and female mate choice.

Materials and Methods

Weights of Field-Collected Adults. To obtain a measure of the variability within field populations of *E. conspersus,* insects were field collected by searching host plants in areas surrounding orchards in Chelan and Douglas counties in Washington state during the period of 25 April through 12 July 2003. Insects were sexed and weighed at the WSU Tree Fruit Research and Extension Center laboratory in Wenatchee, WA, using a digital scale accurate to 0.1 mg (model 200s; Ohaus, Pine Brook, NJ). Each insect was weighed only once, immediately on arrival from the field. The data were normally distributed, and weights of field-collected adults were analyzed using analysis of variance (ANOVA) to detect differences between weights of males and females. A two-factor ANOVA was used to test for interaction between the main effects of date and weight.

Male Weight Change During Mating. Adult *E. conspersus* were field-collected, as outlined above, from areas surrounding orchards in Chelan and Douglas counties. Collection began in late March and was completed by 12 April 2004, immediately after emergence of the insects from overwintering sites. The insects are relatively inactive during this period, but are found basking during warm and sunny days. Dissections using criteria developed by Toscano and Stern (1980) revealed that adult *E. conspersus* are reproductively immature during this period, and previously collected data show that they typically begin mating and oviposition in early to mid-May (Alcock 1971, McGhee 1997). Our early collection periods ensured that insects were gathered while they were still virgins.

Sixty-five male and female pairs of *E. conspersus* were individually placed in 32-oz. transparent plastic cups (Solo Cup Co., Urbana, IL) that had 1-cm-diameter holes drilled into the cup bottoms. These holes allowed for the placement of a cut mullein (*Verbascum thapsus* L.) leaf into the cup, the stem of which would protrude through the bottom of the cup and into a water reservoir below. A mullein leaf was chosen because it provided a natural and familiar perching, mating, and oviposition substrate for the insects (Krupke et al. 2001). Each cup also contained a single green bean (*Phaesolus vulgaris* L.), that was replaced twice each week, as well as a small piece of cardboard with several raw organic sunflower (*Helianthus annuus* L) and peanut (*Arachis hypogaea* L.) seeds glued to its surface. Cardboard seed cards were replaced weekly.

Cups were held at conditions that closely approximated those of the outside environment (unheated, well-ventilated greenhouse) and exposed only to ambient cycles of light and darkness. Cups containing pairs of bugs were monitored hourly throughout the experiment—each monitoring interval consisting of a check of whether insects were mating or not and the identity of the partner. Times that mating commenced and ceased were recorded. All insects were weighed immediately before the experiment, and again after mating, or at 0800 hours if they did not mate during the previous night, and the percentage of weight gained or lost was calculated for each individual. This protocol was followed for 6 consecutive d from 10 to 15 May. Bugs that were known not to have mated were used as controls. A two-factor ANOVA was used to test for interactions between the main factors of sex and mating status. Differences in percentage weight change with respect to number of matings were analyzed for each sex using single-factor ANOVA. The time mating pairs spent in copulae was analyzed using ANOVA as well. Percentage weight change data and copulation times were normally distributed and did not require transformation. Where differences were detected with ANOVA, mean percentage weight changes and copulation times were analyzed using Fisher least significant difference (LSD) test for separation of means (Zar 1999).

Effects of Mating on Longevity and Fecundity. To determine the effects of mating on longevity in adult *E. conspersus,* we placed 30 pairs of virgin, reproductively mature males and females together in 946-ml containers with mullein leaves as described above. Same-sex pairings of 30 virgin males and 30 virgin females (i.e., 15 pairs of each) were placed in identical enclosures during the same period to provide unmated controls. These insects had previously been maintained in same-sex greenhouse colonies with mullein plants, green beans, and organic sunflower seeds and peanuts as food sources. After the outset of the experiment, no other food sources (aside from the mullein leaf) were supplied to the insects, to supply water with only minimal nutrients, thereby simulating an environment where high-quality food sources (i.e., seeds, fruits) were scarce. Insects were held in the same cups for the duration of the experiment, with any dead individuals removed twice weekly. Cups containing male-female pairings were checked nightly until mating was observed. The total numbers of matings over the course of the experiment were not recorded. Data collected included the lifespan of each insect used during the course of the study and the numbers of eggs laid per female per day. This experiment was conducted between 28 May and 13 August 2004. Data were analyzed using two-factor ANOVA to detect interactions between the variables of treatment (mating versus same-sex pairs) and sex with respect to lifespan. Numbers of eggs laid were analyzed using single-factor ANOVA to detect differences between treatments. Data were normally distributed and did not require transformation.

Effect of Size on Mate Choice. To directly assess the influence of size on mate choice, 946-ml mating cups were set up with a cut mullein leaf and supplemented with green beans, peanuts, and sunflower seeds as outlined above. All insects in the colony population were weighed before the experiment. Each cup contained one "large" (upper 25th percentile of colony population weight range) and one "small" (lower 25th
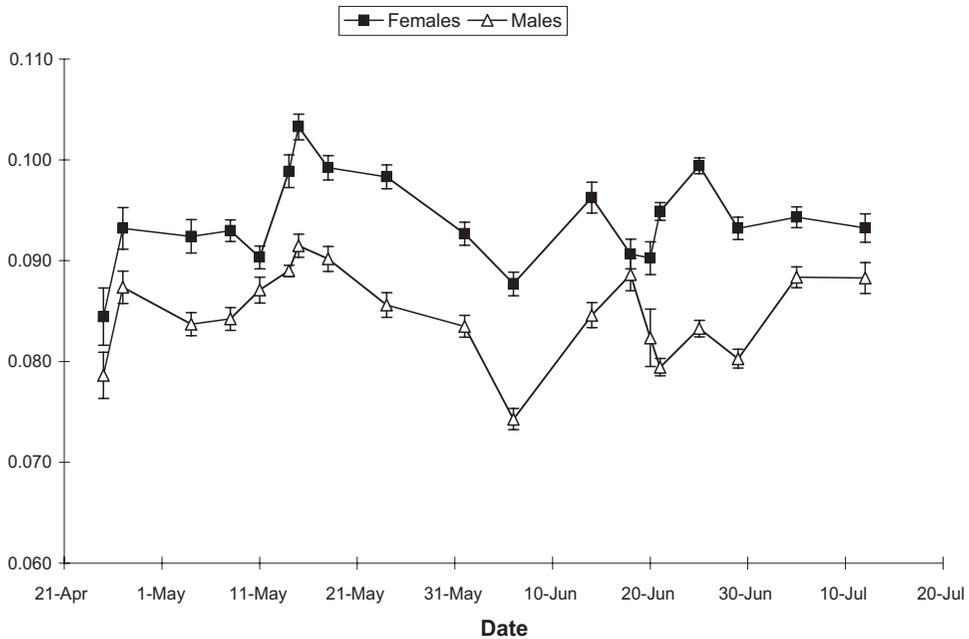
Fig. 1. Mean weights (mg) of field-collected adult male and female *E. conspersus* collected from 25 April to 12 July 2003 from areas surrounding commercial orchards in Chelan and Douglas counties in Washington State.

percentile of colony population weight range) virgin male in combination with a virgin female (within the middle 50% of the colony population weight range) to test the prediction that insects will preferentially mate with large conspecifics. We also performed the same experiment using two females, one each in the upper and lower 25th percentile of weight, with a single male to determine if male choice based on size was occurring. A total of 100 mating trios (50 male-biased, 50 female-biased) were set up in this manner. Individual insects were identified by a small (<2 mm in diameter) nail-polish mark on the scutellum (Super Shine Naturistics; Del Laboratories, Farmingdale, NY). Insects were observed hourly as described in the weight change experiments above. The experiment was run for five consecutive nights (17–21 May 2004) or until a choice was made. Mate choice was recorded in cups where mating was observed. The effect of size on mating success was analyzed using a log-likelihood ratio (Zar 1999) to determine whether there were significant differences in mate preference based on size.

Effect of Experience on Mate Choice. To test the hypothesis that mating experience may influence attractiveness as a potential mate, mating cup experiments were set up using trios consisting of a single, marked virgin female with one marked, experienced male (2 d after previous mating) and one marked virgin male. A reciprocal experiment was conducted using two marked females and one male. All insects used were of the similar weight category within the colony (the middle 50% of the colony weight range). Insects were identified with a small drop of nail polish on the scutellum. A total of 76 mating trios (38 male-

biased and 38 female-biased) were set up in this manner. As in the previous experiment, insects were observed hourly, and the first choice of mating partner was recorded. The experiment was conducted for five consecutive nights (24–28 May 2004), or until a choice was made. The effect of size on mating success was analyzed using a log-likelihood ratio to determine whether there were significant preferences for virgin versus experienced mates.

Results

Weights of Field-Collected Adults. A total of 1,791 females and 1,446 males were weighed in 2003. Females were significantly heavier overall ($F = 838.79$, df $= 1,3235, P < 0.001$). Mean weights ($\pm$SEM) overall were 96.0 ± 0.3 mg for females and 83.2 ± 0.3 mg for males. Analysis using a two-factor ANOVA revealed a significant weight $\times$ date interaction ($F = 12.26$, df $= 3,3762$, $P < 0.001$), with both sexes exhibiting the largest weight gains early in the season shortly after breaking diapause and beginning to feed. Diapausing females (mean $\pm$ SEM) weighed 84.0 ± 2.8 mg ($n = 225$ females), and feeding females weighed 97.1 ± 1.1 mg ($n = 1,556$ females). Diapausing males (mean $\pm$ SEM) weighed 76.0 ± 1.9 mg ($n = 320$ males), and feeding males weighed 85.1 ± 0.8 mg ($n = 1,126$ males; Fig. 1).

Male Contributions During Mating. Of the 65 pairs at the outset of the experiment, 30 mated and 33 did not. Two males died during the course of the experiment, and these mating cups were removed from the analysis. No females laid eggs during the course of this experiment. There was a significant interaction, with

Table 1. Mean percent weight change, actual weight change, and copulation times of *E. conspersus* adults in mating cups when provided with food ad libitum

Number of copulations	Mean preweight (mg) (SEM)		Actual weight change (mg) (SEM)		Mean percent weight change (SEM)		Mean copulation time (h) (SEM)
	Males	Females	Males	Females	Males	Females	
0 ($n = 33$)	104.9 (2.4)	119.4 (1.8)	0.6 (0.9)	−1.2 (1.3)	0.6 (0.9)b	−1.0 (1.1)b	—
1 ($n = 30$)	105.2 (2.5)	120.6 (2.4)	−15.0 (1.1)	6.4 (1.3)	−14.3(1.0)a	5.3 (1.1)a	14.8 (0.6)a
2 ($n = 20$)	98.1 (1.1)	132.6 (2.7)	−1.6 (1.2)	4.0 (1.5)	−1.6(1.2)b	3.0 (1.1)b	12.5 (0.5)b
3 ($n = 12$)	96.8 (1.1)	130.0 (3.9)	−1.1 (1.4)	1.3 (5.1)	−1.1(1.4)b	0.1 (2.8)b	10.4 (0.4)c

Percentage weight change means within each column followed by the same letter are not significantly different (Fisher's LSD multiple comparison test, $P \leq 0.05$).

respect to weight change, between the main factors of the sex of the insects and mating status ($F = 23.05$, df $= 1,186$, $P < 0.001$). Overall, males that mated lost a significantly greater percentage of their body weight (mg) than males that did not mate over the same time period ($−7.6 \pm 1.0$ versus $+0.6 \pm 1.3\%$ for mated and unmated, respectively; $F = 25.17$, df $= 1,93$, $P < 0.001$). There were no significant differences in percentage female weight changes overall ($+1.6 \pm 1.0$ versus $−1.0 \pm 1.0\%$, for mated and unmated, respectively; $F = 2.76$, df $= 1,93$, $P = 0.10$). The first mating resulted in the largest percentage weight loss for males and the largest gain for females (Table 1). The first mating was also significantly longer in duration than subsequent matings (Table 1; $F = 14.02$, df $= 2,47$, $P < 0.05$).

Effects of Mating on Longevity and Fecundity. All individuals in the opposite-sex pairings mated at least once within the first five nights of the experiment. Overall, insects in same-sex pairings lived longer than those in mating pairs ($F = 11.89$, df $= 1,116$, $P < 0.001$; Table 2). This was largely because of the fact that males lived significantly longer in the same-sex pairings than their counterparts who mated ($F = 16.57$, df $= 1,58$, $P < 0.001$). No significant differences were detected in analysis of female lifespans ($F = 0.26$, df $= 1,58$, $P = 0.60$). There were no significant differences in the numbers of eggs laid per female per day between same-sex and mated females ($F = 0.01$, df $= 1,58$, $P = 0.94$; Table 2), aside from the obvious difference that none of the same-sex females produced fertilized eggs.

Effect of Weight on Mate Choice. A total of 44 of 50 males and 34 of 50 females mated over the 10-d observation period. No significant preferences based on size were detected ($G = 0.01$, df $= 1$, $P = 0.90$). Of females making a choice, 19 chose heavier males, whereas 15 chose lighter ones. Of males making a

Table 2. Mean lifespan (d) of adult male and female *E. conspersus* held in pairs with either opposite-sex or same-sex partners and mean numbers of eggs laid per female per day

Treatment	Mean lifespan (d) (SEM)		Mean eggs laid per female/d (SEM)
	Males	Females	
Same sex	37.1 (2.1)a	20.0 (2.4)a	1.2 (0.1)a
Opposite sex	23.1 (2.7)b	18.5 (1.6)a	1.2 (0.1)a

Means within each column followed by the same letter are not significantly different (ANOVA, $P \leq 0.05$) ($n = 30$).

choice, 24 chose heavier females and 20 chose lighter females.

Effect of Experience on Mate Choice. A total of 36 of 38 males and 28 of 38 females mated over the 10-d observation period. There were significant preferences detected based on experience ($G = 16.08$, df $= 1$, $P < 0.001$), and males consistently mated with virgin females ($\chi^2 = 10.02$, df $= 1$, $P < 0.01$), whereas females consistently mated with experienced males ($\chi^2 = 4.32$, df $= 1$, $P < 0.05$).

Discussion

The results of these studies clarified some of the mating dynamics of *E. conspersus.* The finding that the initial mating of *E. conspersus* males resulted in a large weight loss was not surprising in light of work with other stink bug species (Mitchell and Mau 1969, Kon et al. 1993, Wang and Millar 1997). Although the precise nature of these contributions was not identified in our study (or any of the others listed), our experimental results suggest that there is little or no direct benefit of this material to *E. conspersus* females in terms of longevity or fecundity benefits to females that mated compared with those that did not. However, we must note that, because we made no measures of egg quality in the course of these experiments (i.e., fertility or offspring size), we cannot dismiss the possibility of effects at this level.

Although the production of unfertilized eggs by females was not surprising and has been shown in similar experiments with other stink bug species (Mitchell and Mau 1969, Youther and McPherson 1975, Wang and Millar 1997), it was interesting to note that female *E. conspersus* in our study produced an almost identical number of eggs as their mated counterparts. This pattern of equal lifespan and fecundity in mated versus unmated females has been shown in other insect species (Reguera et al. 2003) and reinforces the possibility that sperm is the only useful component of the material transferred by the male and that this material is not used as a nutritional source by female *E. conspersus.*

The observation that females exhibited no mating biases associated with male weights in our experiments is useful in identifying cues for mate choice in this species. If females gained a direct, material benefit from mating, it may be expected that they would mate

more often with larger males when given the choice, and large male choice has been shown by the work of other researchers working within the Pentatomidae (McLain 1980, Capone 1995). The main difference between our studies and those of other researchers within the Pentatomidae are that the studies described above used constant and linear measurements of adult size (dorsal surface area and pronotal width, respectively) to assign size categories, whereas our study used weight to test hypotheses about direct, material benefits to females associated with male size. Furthermore, size alone may not be a reliable indicator to potential mates. That is, although large males may be more likely to transfer more material when mating, a smaller virgin male may transfer more material than an experienced, larger one.

Our experimental tests of the prediction that females would choose to mate with virgin males (based on the potential for largest percentage weight gains) complement results discussed in the previous paragraph—females actually mated with experienced males more often than virgins—suggesting that potential male weight contributions cannot be assessed a priori by the female.

Courtship intensity can be a powerful factor influencing female mate choice in polyandrous species (Hosken et al. 2003), and may have been an overriding factor in these experiments. Courting by antennation, head-butting, and acoustical signals has been documented in *E. conspersus* (Alcock 1971, McBrien and Millar 2003), and we witnessed evidence of these behaviors during our observations, although these variables were not quantified during our study. These nonvisual courtship cues are probably important in *E. conspersus*, a species that mates primarily at night, during hours of full darkness (Krupke et al. 2005), making visual assessments of size difficult for prospective mates. Our results do not exclude the possibility that *E. conspersus* females prefer males that are larger in size, but they do indicate that females do not discriminate based on the potential weight transfer from prospective partners.

Overall, the absence of longevity and fecundity benefits, combined with the absence of a large male or virgin male mating advantage, indicates that a large male weight contribution has no apparent direct value to females and is not a critical aspect in female mate choice. We also did not address the possibility that the transfer of material may act as a mating plug, however. Such a function would potentially benefit the males, and, although this hypothesis has not been tested in the Pentatomidae, mating plugs have been shown in a variety of taxa, including Hymenoptera, Diptera, and Orthoptera (Woyciechowski et al. 1994, Polak et al. 1998, Yi Shu and Gillott 1999). All of these insects exhibit polyandry, as is frequently the case in the Pentatomidae, including *E. conspersus* (Alcock 1971).

Whatever its purpose, the production of the material transferred to the female is clearly energetically costly—there was a longevity cost associated with mating for males. In light of other published work on stink bugs, male choice of virgin females in our ex-

periments was not surprising, particularly if sperm precedence patterns follow those of *N. viridula*, where there is increased fertilization efficiency for the first male to fertilize a multiply-mated female (McLain 1985). This also supports "mating plug hypothesis" of explaining the function of weight transfer from males to females.

At this point, it is worthwhile to draw attention to the fact that our colony-maintained insects may have benefited from ad libitum access to food and water in the laboratory while they matured reproductively. This may have led to a greater difficulty in stressing these individuals during longevity studies and perhaps obscured potential benefits of mating to the females. It has been shown in other insects that the longevity benefits of multiple mating are only evident under harsher environmental conditions (Fox 1993) and that males providing nutrients are more sought after when conditions are poor (Gwynne 1990). In mate choice experiments, the condition of the insects used may have resulted in reduced choosiness because both potential mates in any given trio were heavier than those normally encountered in the field.

Our laboratory experiments yielded no evidence to support our hypothesis of male mate choice in *E. conspersus*. Although males make an energetically costly body weight contribution during mating, we did not show any fecundity or longevity benefits to females as a result. In light of our data, future experiments should examine the hypothesis that transferred materials act to increase male fertilization efficiency in some way.

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