

AN ASSESSMENT OF THE EFFECTS OF SEXUAL SELECTION AND THE
SOCIAL ENVIRONMENT ON MATING BEHAVIORS IN TWO TIGER
BEETLE SPECIES (COLEOPTERA: CICINDELIDAE)

THESIS

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS..	iv
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
ABSTRACT.....	xi
CHAPTER	
I: Effects of the Social Environment on Mating Behaviors.	1
INTRODUCTION	1
Objectives.....	4
MATERIALS AND METHODS.....	5
Study Sites.....	5
Data Collected.....	6
Laboratory Experiments.....	6
Experiment 1: Mating Constraints (1 Male· 1 Female).....	7

Experiment 2: Male Choice (1 Male: 2 Females).....	7
Experiment 3: Female Choice	8
Experiment 4: Male-Male Competition (2 Males: 1 Female).....	9
Effects of the Social Environment: Intraspecific and Interspecific Comparisons.....	11
RESULTS.....	12
Experiment 1: Mating Constraints (1 Male: 1 Female).....	12
Experiment 2: Male Choice (1 Male: 2 Females).....	13
Experiment 3: Female Choice.....	14
Experiment 4: Male-Male Competition (2 Males: 1 Female).....	14
Effects of the Social Environment: Intra and Interspecific Comparisons.....	17
<i>Intraspecific</i>	17
<i>Interspecific</i>	17
DISCUSSION.....	18
Experiment 1: Mating Constraints (1 Male: 1 Female).....	20
Experiment 2: Male Choice (1 Male: 2 Females).....	21
Experiment 3: Female Choice.....	22
Experiment 4: Male-Male Competition (2 Males: 1 Female).....	23
Body Size Correlations.....	25
Effects of the Social Environment: Intra and Interspecific Comparisons.....	26

<i>Intraspecific</i>	26
<i>Interspecific</i>	27
Conclusions.....	29
Future Studies.....	30
References.....	43

II SIZE ASSORTATIVE MATING AND SEXUAL SIZE

DIMORPHISM.....	46
INTRODUCTION.....	46
Size Assortative Mating.....	47
Sexual Size Dimorphism..	49
Mate Guarding	50
Study System.....	53
Objectives.....	53
MATERIALS AND METHODS.....	55
Study Sites.....	55
Data Collected.....	55
Observational Experiment 1: Assortative Mating in the Field.....	55
Observational Experiment 2: Sexual Size Dimorphism....	56
Observational Experiment 3: Mate Availability.....	56
Observational Experiment 4: Mating Behavior Sequence..	57
RESULTS.....	57

Observational Experiment 1: Assortative Mating in the Field.....	57
Observational Experiment 2: Sexual Size Dimorphism....	58
Observational Experiment 3: Mate Availability.....	58
Observational Experiment 4: Mating Behavior Sequence.....	59
<i>Cicindela belfragei</i> and <i>C. formosa</i> Mating Behavior Characteristics.....	59
Male-Male Contests..	61
DISCUSSION.....	62
Observational Experiment 1: Assortative Mating in the Field	62
Observational Experiment 2: Sexual Size Dimorphism....	63
Observational Experiment 3: Mate Availability..	65
Observational Experiment 4: Mating Behavior Sequence..	65
Why Should Male Choice and Male-Male Competition Not Coincide?.....	66
Conclusions.....	67
Future Studies..	67
References.....	69

LIST OF TABLES

Table	Page
1 Results of <i>C. belfragei</i> Pearson's correlation tests between guarding and copulation durations and male and female size in the three mating experiments.....	32
2 Results of <i>C. formosa</i> Pearson's correlation tests between guarding and copulation durations and male and female size in the three mating experiments.....	34
3 Mean guarding and copulation durations of <i>C. belfragei</i> and <i>C. formosa</i> males in the three mating experiments.....	36

LIST OF FIGURES

Figure	Page
1	Positive correlation between female size and copulation duration in the <i>C. belfragei</i> male choice experiment.....37
2	Positive correlation between female size and guarding duration in the <i>C. formosa</i> mating constraints experiment.....37
3	Positive correlation between female size and copulation duration in the <i>C. belfragei</i> male choice experiment.....38
4	Negative correlation between male size and copulation duration in the <i>C. formosa</i> male choice experiment.....38
5	Positive correlation between male size difference and guarding duration in the <i>C. belfragei</i> male-male competition experiment.....39
6	Positive correlation between male size difference and the number of attacks on mating males in the <i>C. belfragei</i> male-male competition experiment.....39

7	<i>C. belfragei</i> and <i>C. formosa</i> boxplots; male strength of preference (SOPm) for large females in the male choice experiment, female strength of preference (SOPf) for large males in the female choice experiment, and strength of mating (SOM) by large males in the male-male competition experiment.....	40
8	Bar chart of mean guarding and copulation durations in the <i>C. belfragei</i> mating experiments.....	41
9	Bar chart of mean guarding and copulation durations in the <i>C. formosa</i> mating experiments .	42

ABSTRACT

AN ASSESSMENT OF THE EFFECTS OF SEXUAL SELECTION AND THE SOCIAL ENVIRONMENT ON MATING BEHAVIORS IN TWO TIGER BEETLE SPECIES (COLEOPTERA: CICINDELIDAE)

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Mate guarding is a sexually selected behavior performed by males of many species that results due to sperm competition. Facultative mating behaviors are predicted to occur in these taxa when changes in the social environment alter the benefits for

guarding males. I used field observations and lab experiments in 2006 and 2007 to investigate the factors influencing mating behaviors in two tiger beetle species that exhibit mate guarding behavior in central Texas, *Cicindela belfragei* and *C. formosa*. Mate guarding and copulation durations were affected by female size, indicating that males may be capable of assessing female size once in physical contact with the female. The social environment of *C. belfragei* experiments did not affect guarding or copulation durations, but in *C. formosa*, social context was important. In *C. formosa*, mating behavior durations (guarding and copulation durations) were greatest when only one female was available, but were also longer when non-mating males were in the mating area, compared to when females were abundant.

Size assortative mating and female-biased sexual size dimorphism (SSD) are expected to occur in taxa with post-copulatory mate guarding due to selection for male choice of large females when male time investment is high. Both species displayed female-biased SSD, but size assortative mating did not occur in the field. However, the mean size of mating *C. belfragei* females collected in the field in 2006 was larger than the mean size of non-mating females, suggesting that large females may have a mating advantage. The results of this study suggest that male choice of large females does not cause the female-biased SSD in these species, assortative mating may not be a characteristic of these species, and that mating behavior durations may not be affected by the extent of mating male harassment by non-mating males or by the outcome of male contests for females. Instead, this study supports previous findings, that *Cicindela* mating behavior may be determined by other factors, such as the location of oviposition sites and the ability to fly.

CHAPTER I

Effects of the Social Environment on Mating Behaviors

INTRODUCTION

Darwin (1859) introduced the term sexual selection to explain certain behavioral and morphological traits assuming that competition among males was strictly precopulatory. Parker (1970) revealed that sexual selection can also occur after mating through sperm competition. He suggested that selection should favor behaviors and morphological structures that improve a male's ability to defend his own sperm, and also manipulate a rival's sperm.

Mate guarding is an important sexually selected behavior that results due to sperm competition. Mate guarding may increase the number of offspring sired by males, and is a commonly observed behavior in insects (Thornhill and Alcock 1983, Alcock 1994, Simmons 2001). Some characteristics of a species' mate guarding will depend on its biology. For example, last sperm precedence, which occurs when the sperm from the last

mating is used to fertilize most of the eggs, may result in selection for post-copulatory mate guarding behavior (Parker 1970). However, aspects of mate guarding such as the length of time a male spends paired with a particular female may change according to more variable conditions such as sex ratios, density of conspecifics, the availability of mated females, and the quality of available females.

Theoretical studies have investigated how post-copulatory mate guarding may have evolved and how different social environments should affect mate guarding behavior (Parker 1970, Parker 1974, Yamamura 1986, Alcock 1994). Parker (1974) considered time spent mating as a cost of mate guarding and found that post-copulatory mate guarding should be favored when the sex ratio is male-biased, due to an increase in the probability of multiple matings by the female. Yamamura (1986) predicted that mate guarding should benefit males when the sex ratio is male biased, when the density is high, and when the female lays eggs soon after copulation. Finally, Alcock (1994) suggested several conditions for the evolution of mate guarding, including last male advantage, high potential of taking over of previously mated females, and a male-biased sex ratio.

These theoretical predictions have been tested empirically in numerous studies (e.g. Manning 1980, McLain 1982, Alcock 1991, Brown and Stanford 1992, Shivashankar and Pearson 1994, Saeki et al. 2005). For example, Alcock (1991) confirmed Yamamura's prediction of the effects of female availability and high intensity of sperm competition in a staphylinid beetle *Ontholestes cingulatus*. Also, Stoks et al. (1997) demonstrated that the mate guarding behavior of the emerald damselfly, *Lestes sponsa*, supported the 10 predictions of Alcock (1994) for the evolution of mate

guarding, including a high density, a male-biased operational sex ratio, a high male capacity to resist takeovers, high female receptivity after copulation, high access by rivals to mated females, and a short interval between copula and oviposition.

Tiger beetles are an excellent system for examining factors that may affect mating behaviors. These predatory arthropods are found all over the world except Tasmania, Antarctica, and remote oceanic islands (Pearson 1988). Tiger beetles are well suited for tests of mate guarding and sexual selection hypotheses because male and female tiger beetles mate with multiple partners (Kraus and Lederhouse 1983, Fielding and Knisley 1995, Rodriguez 1998). Copulation usually occurs within the first few minutes after the male mounts the female (Palmer 1976, Kraus and Lederhouse 1983, Shivashankar and Pearson 1994, Fielding and Knisley 1995, Rodriguez 1998). In many tiger beetle species males remain attached to females without genital contact after copulation, lasting from a few minutes to several hours, which has widely been interpreted as mate guarding behavior (Pearson 1988). Females of some species have been observed depositing eggs in the soil with males still attached (personal observation). Interestingly, in the lab males often terminate guarding apparently prior to oviposition. However, male behavior while in amplexus, such as fights with non-mating males and takeovers of mating males by non-mating males, suggest that post-copulatory amplexus has a guarding function (Kraus and Lederhouse 1983, Shivashankar and Pearson 1994, Fielding and Knisley 1995, Rodriguez 1998).

In tiger beetles there is a paucity of information on factors that may affect the availability of mated females such as the delay between copulation and oviposition and the distance from the mating area to the oviposition site. The intensity of sperm

competition is likely high in tiger beetles, since a male-biased sex ratio has been observed in several species (Shivashankar and Pearson 1994), and there is indirect evidence for sperm precedence, given by the copulatory stages (Fielding and Knisley 1995, Rodriguez 1998) and aedeagus morphology (Freitag et al. 1980). Also, in one tiger beetle species the body length of mating pairs observed in the field was significantly correlated (Kraus and Lederhouse 1983), indicating that larger mates may be preferred. Accordingly, in this study the sex ratio in the mating area, sizes of males and females, and the potential for mate choice were examined to determine if the social environment affects the mate guarding duration of two tiger beetle species, *Cicindela* (Dromochorus) *belfragei* and *C.* (Dromochorus) *formosa* from central Texas.

Objectives

This study was designed to assess how a male's immediate social environment may affect mate guarding duration in two tiger beetle species. To evaluate how the social environment may affect mate guarding behavior, I asked the following questions:

1. Are there physical constraints on mate pairing?

If any size combinations of males and females have physical difficulties in pairing, then mating constraints may affect mate guarding.

2. Can males exert mate choice, and if so, do they prefer large females?

If males prefer to mate with large females, then male choice may affect mate guarding.

3. Can females exert mate choice, and if so, do they choose large or small males?

If females prefer large or small males, then female choice may affect mate guarding.

4. Does male-male competition occur and impact mate guarding?

If large males have a mating advantage in male contests then this may affect mate guarding.

5. Does the social environment affect mate guarding?

Selection should favor males capable of adjusting mate guarding duration when the social environment alters the cost-benefit ratio of mate guarding.

The results of these experiments should clarify size biases in each component of mating and shed light on the processes of sexual selection responsible for the mating pattern observed. A comparative analysis of the results of these experiments also gives insight into the different behaviors and ecological factors that may affect mate guarding in these two species of tiger beetles.

MATERIALS AND METHODS

Study Sites

To evaluate mate guarding in *C. belfragei*, individuals were collected on private property in the Edwards Plateau near San Marcos, Texas. *Cicindela belfragei* are flightless and were easily collected by placing a plastic container in front of them as they attempted to evade capture. *Cicindela belfragei* were collected in open areas with sparse vegetation and relatively abundant soil at least once a week from May through early July 2006 and again in 2007, the period of mating activity for this species. To investigate the mating characteristics of *C. formosa*, I visited the Griffith League Ranch and Texas State's Welsh property in the Lost Pines near Bastrop, Texas approximately once a week from March through May 2006 and 2007. This is the period of mating activity for *C.*

formosa (Schultz 1989). *Cicindela formosa* are agile fliers and were observed and collected with a butterfly net in open areas of sand in firebreaks and on abandoned roads. The single *C. belfragei* collection site (less than 400 m²) was much smaller in size than the *C. formosa* collection sites (more than 1600 m²). Also, female *C. belfragei* were observed ovipositing in the mating area after amplexus, but females of *C. formosa* were observed leaving the mating area prior to oviposition, suggesting that they utilize sites other than the mating area for oviposition.

Data Collected

The location, date, sex, and size (left elytron length) of all beetles collected was recorded with a digital caliper. Elytron length correlates with body weight in many beetle species, and thus is a good estimate of overall size (Kraus and Lederhouse 1983, Brown and Stanford 1992). Mating pairs in the field were also collected, and the size (left elytron length) of each individual in the pair was documented. Beetles collected in the field were placed individually in numbered plastic containers (5 cm wide and 5 cm deep) and were used in experiments in the field on the day they were collected. The mean size of male and female *C. belfragei* and *C. formosa* and the standard deviation of these means determined from the collections were used as a basis for designating large (>1 standard deviation above the mean) and small (>1 standard deviation below the mean) males and females used in the laboratory experiments.

Laboratory Experiments

For each laboratory experiment, I documented the size (left elytron length) of individuals in each trial, copulation duration (genital contact), guarding duration

(amplexus without genital contact), total mating duration (total duration of amplexus), resistance behaviors by females (kicking, shaking, rolling), and attacks and removals of mating males by non-mating males in the male-male competition experiments. Each beetle was used for one experiment per day, and no individuals were used in the same experiment more than once. Pairs that did mate were observed until 10 minutes after termination of amplexus. All experiments took place in a 25x50x30 cm aquarium divided in half (each chamber was 12.5 cm wide by 25 cm long). All beetles were marked with a silver Sharpie® pen to indicate the experiment in which they were used, then at the end of each day they were released where they had been collected.

Experiment 1: Mating Constraints (1 Male: 1 Female)

To evaluate mating behaviors when only a single male and female are in the mating area, I placed small, average, and large males with small, average, and large females a pair at a time in the aquarium and recorded all mating behaviors (N = 33 *C. formosa*, N = 31 *C. belfragei*). Pearson's product moment correlation tests were used to determine if female or male size influenced guarding or copulation durations. Due to the fact that larger females are likely to be more fecund (Thornhill and Alcock 1983), I expect longer guarding durations with larger females. Therefore, in each mating experiment two-tailed tests were used for male size correlations, and one-tailed tests were used for female size correlations.

Experiment 2: Male Choice (1 Male: 2 Females)

To determine how two females available for one male affects mate guarding, and to determine if males choose mates based on size, for each species I placed an average

sized male in the aquarium with a small and large female and documented all mating behaviors ($N = 22$ *C. belfragei*, $N = 31$ *C. formosa*). Again, I used Pearson's product moment correlation tests to determine if female or male size influenced guarding or copulation durations. To perform these correlation tests I calculated the mean mating behavior durations and mean female size for the trials in which both females mated. Also, if males have no preference for large versus small females then I expect an equal number of matings with small, large, and both large and small females (1 1:1). Therefore, I used X^2 goodness-of-fit tests to look for significant deviation from these expected mating frequencies. In both species the male strength of preference (SOPm) for large or small females was calculated within each trial by dividing the amplexus duration with a large female by the sum of amplexus durations within each trial ($SOP > 0.5$ potentially stronger preference for large female, $SOP < 0.5$ potentially stronger preference for small female). In trials where males mated with both females, X^2 goodness-of-fit tests were used to look for significant differences between which female the male mated with first.

Experiment 3: Female Choice

To determine if female choice of males may influence mate guarding duration, for each species I placed a female in the aquarium with a small and a large male ($N = 17$ *C. belfragei*, $N = 33$ *C. formosa*). Each male was placed in a clear plastic petri dish (9 cm diameter) with fifteen 3mm holes drilled in the lid. These dishes were centered on opposite ends of the aquarium (12 cm by 15 cm by 25 cm mating chamber), each 10 cm from their respective end of the tank. The side of the mating chamber in which the large male was placed was alternated between each trial. The female was observed for 10

minutes and the amount of time spent within one body length of each petri dish was documented. After 10 minutes the Petri dishes were removed and washed with water, freeing the two males (initiating Experiment 4).

The female strength of preference (SOP) for large males was determined by dividing the amount of time a female spent within one body length of the large male by the total amount of time a female in that trial spent within one body length of both Petri dishes. A box plot was generated from the SOP data (mean SOP < 0.5 potentially stronger preference for small males, mean SOP > 0.5 potentially stronger preference for large males). For *C. belfragei* two-tailed paired *T*-tests were used to compare the mean amount of time females spent within one body length of small and large males in the petri dishes. Due to heteroscedastic variances in the *C. formosa* female choice data, Wilcoxon signed-rank tests were used to look for significant differences in female behaviors. If females spend more time near large or small males in the Petri dishes then female choice may affect mate guarding duration due to female behaviors favoring large or small males.

Experiment 4: Male-Male Competition (2 Males: 1 Female)

To determine how mate guarding may be affected when two males compete for a single female, the males in Experiment 3 were released from the Petri dishes and the mating behaviors of the female and two males were observed until 10 minutes after termination of amplexus. If large males do not have a mating advantage then I expect an equal number of matings by large, small, and both large and small males in this experiment. Deviation from this 1:1:1 expected mating frequency was evaluated using χ^2 goodness-of-fit tests. Also, the number of attacks or bouts on mating males, and the

number of removals of mating males was assessed for divergence from the expected random frequency of 1:1 using X^2 goodness-of-fit tests. Pearson's correlation tests were used to evaluate whether male and female size affected mating behaviors.

In this experiment the strength of males (SOM) was the large male amplexus time divided by the total amplexus duration by both males within each replicate. A box plot was generated to show the distribution of SOM values, and the mean SOM was calculated for each species (mean SOM < 0.5 potentially stronger mating or longer amplexus by small males, mean SOM > 0.05 potentially stronger mating by large males). The number of attacks on mating males, and the number of removals of mating males was assessed for divergence from the expected random frequency of 1:1 using X^2 goodness-of-fit tests. Also, for trials in which both males mated, X^2 goodness-of-fit tests were used to look for significant differences between which male mated first.

Mean male size and mean guarding and copulation durations were calculated in the trials where both small and large males mated. Wilcoxon signed-rank tests were used to look for differences in large versus small male mating behavior durations in the *C. belfragei* and *C. formosa* trials in which both males mated successfully. These non-parametric tests were used because of small sample sizes and deviations from normal distribution of data in both species. For the trials in which both males mated, the difference in size between large and small males was tested for correlations with guarding and copulation duration and number of attacks and removals of mating males using two-tailed Pearson's correlation tests. Also, logistic regression was used to evaluate the affect of mating male size relative to female size (male size/female size) on takeover rate to determine if males that are much smaller than the female are more likely

to be displaced by non-mating males. Finally, differences in mating characteristics in the absence of male–male competition (experiments 1 and 2) were compared to behaviors in this experiment with two-sample *T*-tests to evaluate the potential for plasticity in male mating behaviors among these two tiger beetle species.

Effects of the Social Environment: Intra and Interspecific Comparisons

To investigate whether the social environment influences mate guarding, I compared the mean duration of each mating behavior among the three mating experiments for each species. If different conditions in the social environment affect the costs and benefits of male mating behaviors, then selection should favor males that adjust these behaviors to achieve the optimal time investment strategy. Since multiple experiments measured mating male guarding and copulation durations within each species, I first used single factor ANOVAs and MANOVAs to evaluate the effect of the type of experiment on these mean mating behavior durations in *C. belfragei* and *C. formosa*. Second, Welch modified two sample *T*-tests were used to look for all pairwise differences in mating behaviors within the *C. belfragei* and *C. formosa* experiments. Third, Welch modified two sample *T*-tests were used to look for differences in mating behaviors between the two species. Fourth, the extent of mating male harassment by non-mating males in the male-male competition experiment was calculated for each species (attacks/minute amplexus). This provides an estimate of the level of male competition in each species and allows comparison of the relative strength of male competition between the two species. Due to a non-normal distribution of data, a Wilcoxon rank-sum test was used to test for significant difference between the extent of male harassment observed in each species. Fifth, the operational sex ratio in the mating

area on each collection date was calculated by dividing the number of males collected each day by the total number of beetles collected that day (< 0.5 indicates more females than males, > 0.5 indicates more males than females). This operational sex ratio estimate was evaluated for bias by calculating the 95% confidence interval of the mean value for each species. Lastly, the density of individuals found in the collection areas was estimated by calculating the number of beetles captured per hour of searching. This provides an estimate of the relative abundance of *C. belfragei* and *C. formosa* in the mating area. Evaluating mating behavior differences within and between *C. belfragei* and *C. formosa* experiments provides a basis for understanding the various ecological, social environment, and mating system characteristics responsible for these differences.

RESULTS

Experiment 1: Mating Constraints (1 Male: 1 Female)

All pairs of *C. belfragei* mated successfully when a single male and female were placed in the mating chamber. There was a significant positive correlation between female size and duration of copulation in *C. belfragei* ($r = 0.496$, $df = 28$, $t = 3.02$, $p = 0.0026$, Fig 1). However, there was no significant correlation between female size and guarding duration (Table 1). Also, male size did not correlate with guarding or copulation duration (Table 1).

All *C. formosa* pairs in the mating constraints trials mated. There was a significant positive correlation between female size and guarding duration ($r = 0.29$, $df = 31$, $t = 1.69$, $p = 0.05$; Fig 2). However, there was no correlation between female size and

copulation duration, nor was there an effect of male size on guarding or copulation duration (Table 2).

Experiment 2: Male Choice (1 Male: 2 Females)

Of the 22 male *C. belfragei* choice trials, five males mated only with the small female, 10 males mated only with the large female, and 7 males mated with both the large and the small female. There was no significant deviation from the expected frequency if mating was random (goodness-of-fit test; $X^2 = 1.736$, $df = 2$, $p > 0.25$). There was a positive correlation between female size and copulation duration in the *C. belfragei* male choice trials ($r = 0.418$, $df = 20$, $t = 2.1$, $p = 0.0265$; Fig 3). However a more conservative estimate using a two tailed test found no correlation of guarding and female size ($p = 0.053$). There were no significant correlations between female size and guarding duration, nor between male size and guarding or copulation duration (Table 1). In trials where both females mated, there was no difference between which female mated first in the *C. belfragei* male choice experiments (large male first = 2, small male first = 2). The mean SOPm for female *C. belfragei* was 0.65 ± 0.08 (Fig 7).

Of the 31 *C. formosa* trials with one male and two females, 12 males mated with only the small female, 15 males mated with only the large female, and 4 males mated with both the large and the small female, but there was no significant deviation from the expected random mating frequency (goodness-of-fit test; $X^2 = 5.71$, $df = 2$, $p > 0.05$). There was a significant negative correlation between *C. formosa* male size and copulation duration in the male choice trials ($r = -0.41$, $df = 29$, $t = -2.42$, $p = 0.011$; Fig 4), indicating that small males may invest more energy in females. All other tests found no

significant correlations between male or female size and guarding or copulation duration (Table 2). The mean SOP_m for *C. formosa* females was 0.55 ± 0.09 in this experiment (Fig 7).

Experiment 3: Female Choice

There was no significant difference between the mean amount of time the female spent within one body length of the large (mean = 16 sec) and small males (mean = 23 sec) in *C. belfragei* (*T*-test; *df* = 21, *t* = 1.35, *p* = 0.189). The mean SOP_f for *C. belfragei* males was 0.472 ± 0.08 (Fig 7).

A Wilcoxon signed-rank test was used to evaluate the *C. formosa* female choice data due to heteroscedastic variances, but there was no significant difference in the mean amount of time females spent within one body length of large (mean = 52 sec) or small males (mean = 62 sec) in the Petri dishes (*N* = 39, *Z* = 1.88, *p* = 0.06). The mean SOP_f for *C. formosa* was 0.40 ± 0.04 (Fig 7).

Experiment 4: Male-Male Competition (2 Males: 1 Female)

For *C. belfragei* both males mated in 13 trials, in 2 trials only the small male mated, and in 2 trials only the large male mated. These results deviated significantly from the expected random mating frequency of 1:1:1 (goodness-of-fit test; $X^2 = 9.3$, *df* = 2, *p* < 0.01). This was due to both males mating in more trials than expected, indicating that females mate with more than one male. In 5 of 30 attacks by large *C. belfragei* males on small mating males, and in 4 of 39 attacks by small males on large mating males, the mating male was dislodged. The observed attacks and removals of mating males did not differ from the expected random frequencies of 1:1 (goodness-of-fit tests; *p* > 0.25). This

indicates that large males did not attack or remove small mating males significantly more often than small males attacked and removed large mating males. For the 13 trials in which both males mated, the size difference between the large and small male was positively correlated with guarding duration ($r = 0.59$, $df = 11$, $t = 2.44$, $p = 0.033$; Fig 5). Also, there was a positive correlation between male size difference and the number of attacks or bouts on mating males ($r = 0.62$, $df = 11$, $t = 2.6$, $p = 0.024$; Fig 6), but the number of bouts that resulted in removal of the mating male did not correlate significantly with male size difference ($r = 0.43$, $df = 11$, $t = 1.56$, $p = 0.147$). Results of the logistic regression of mating male size divided by mating female size and number of takeovers indicated that male size relative to female size did not affect takeover rate ($N = 13$, t ratio = 1.1, $p = 0.29$). The mean SOM in the *C. belfragei* male-male competition experiment was 0.48 ± 0.08 (Fig 7). In the *C. belfragei* trials in which both males mated, there were no significant differences in guarding (small mean = 70.5 ± 4.2 minutes, large mean = 32 ± 8.0) or copulation (small mean = 26.6 ± 5.4 minutes, large mean = 22.7 ± 4.2) durations between large and small males (Wilcoxon signed-rank tests; $N = 13$, $p > 0.5$). Also, there was no significant difference between which male mated first in *C. belfragei* (goodness-of-fit test; $X^2 = 0.33$, $p > 0.25$), indicating again that large males do not have a mating advantage. Female size did not correlate with guarding or copulation duration in *C. belfragei* ($N = 17$, $p > 0.5$; Table 1).

In the male-male competition experiment with *C. formosa*, both males mated in 14 trials, in 11 trials only the small male mated, and in 8 trials only the large male mated. These mating frequencies were not significantly different than the expected random mating frequency of 1:1:1 (goodness-of fit test; $X^2 = 1.64$, $df = 2$, $p > 0.25$). In *C.*

formosa trials in which both males mated, there were no significant differences between large and small male guarding (small mean = 4.0 ± 2.1 minutes, large mean = 7.9 ± 3.5) or copulation (small mean = 5.0 ± 1.0 minutes, large mean = 4.8 ± 0.47) durations (Wilcoxon signed-rank tests; $N = 14$, $p > 0.25$), nor was there a significant difference between which male mated first (goodness-of-fit test; $N = 14$, $X^2 = 0.28$, $df = 1$, $p > 0.25$). There were 69 attacks by large males on small mating males, 10 resulting in removal of the mating male. There were 54 attacks by small males on large mating males, 8 of which resulted in removal of the mating male. These observed attacks and removals did not differ significantly between the large and small males (goodness-of-fit tests; $p > 0.15$). For the trials in which both males mated, there was no correlation of male size difference and guarding ($r = 0.32$, $df = 12$, $t = 1.14$, $p = 0.27$), copulation ($r = -0.07$, $df = 12$, $t = -0.25$, $p = 0.81$), attacks on mating males ($r = 0.07$, $df = 12$, $t = 0.25$, $p = 0.81$), or the number of removals of mating males ($r = -0.15$, $df = 12$, $t = -0.54$, $p = 0.6$). Also, results of the logistic regression of mating male size divided by mating female size and number of takeovers indicated that male size relative to female size did not affect takeover rate ($N = 14$, t ratio = 0.074 , $p = 0.79$). These results indicate again that large males do not have a mating advantage. In the male-male competition trials using *C. formosa*, neither female size nor male size correlated with guarding or copulation duration (Table 2), these results differ from the observed behavior of solitary males (see experiments 1 and 2), indicating that male-male competition may alter male ability to adjust mating behaviors based on the size of females.

Effects of the Social Environment: Intra and Interspecific Comparisons

Intraspecific

For *C. belfragei*, there was no significant effect of experiment type on guarding or copulation durations (single factor ANOVAs; $p > 0.4$; Table 3, Fig 8). In contrast, all pairwise comparisons of guarding and copulation durations among *C. formosa* experiments were significantly different (Table 3, Fig 9). The social effect on mating behavior duration in *C. formosa* was confirmed with a single factor MANOVA, with experiment type significantly influencing guarding and copulation duration (guarding: $df = 2$, $F = 14.3$, $p < 0.0001$; copulation: $df = 2$, $F = 6.6$, $p < 0.01$). Male *C. formosa* guarding and copulation durations were longer in the mating constraints experiment than in the male choice and the male-male competition experiments (Table 3). The duration of guarding and copulation was longer in the *C. formosa* male-male competition experiment than in the male choice experiment (Table 3, Fig 9).

Interspecific

In all *C. belfragei* experiments male guarding and copulation durations were of significantly longer duration than those of males in the *C. formosa* experiments (*T*-tests; $p < 0.05$; Table 3), suggesting that selection for longer pairing duration is stronger in *C. belfragei* than in *C. formosa*. The mean number of *C. belfragei* collected per minute of searching was greater than that of *C. formosa* (*C. belfragei* = 0.33 ± 0.04 beetles/min, *C. formosa* = 0.23 ± 0.05 beetles/min). The operational sex ratio (OSR) was not male-biased throughout the collection dates in both species (*C. belfragei* mean OSR = 0.58, 95% CI = 0.48 - 0.67; *C. formosa* mean OSR = 0.51, 95% CI = 0.45 - 0.58). The extent

of mating male harassment by non-mating males in the male-male competition experiments (*C. belfragei* = 0.038 ± 0.02 bouts/min amplexus, *C. formosa* = 0.21 ± 0.07 bouts/minute amplexus) did not differ between the two species (Wilcoxon rank-sum test; $Z = -0.99$, $p = 0.16$).

DISCUSSION

Although male and female *C. belfragei* did not show significant preferences for large or small mates (experiments 2 and 3) and male size did not predict success in male-male contests (experiment 4), the mean copulation duration was affected by female size when single males had the opportunity to mate with one female and when single males had a choice of two females (experiments 1 and 2). On the other hand, when two male *C. belfragei* competed for a single female, neither male size nor female size predicted mating behavior durations (experiment 4). Thus, *C. belfragei* males may be able to assess female size and increase copulation duration with larger females when non-mating males are absent in the mating area.

As observed in *C. belfragei*, male and female *C. formosa* did not show preference for large versus small mates. However, male *C. formosa* guarded larger females longer when no other males were present and only one female was available (experiment 1). When males of *C. formosa* had the choice of two females (experiment 2), smaller males copulated significantly longer, but female size did not affect mating behavior durations. When two males of *C. formosa* competed for a single female neither male nor female size affected mating behavior durations, likely due to frequent interruptions by non-mating males (experiment 4). These findings indicate that when only one male is in the mating

area, males may be capable of adjusting mating behaviors as part of an optimal time investment strategy. However, when two males compete for a single female, male competition may prevent mating males from achieving optimal mating behavior durations. Recent work on communication networks has made a strong case for examining the influence of social interactions on mating behavior (Dzieweczynski et al. 2005, Saeki et al. 2005). Results of this study provide evidence that the social environment affects the duration of mating behaviors in *C. belfragei* and *C. formosa*. This is due to the fact that changes in the social environment are likely to have a great impact on the relative benefits of a particular mating strategy (Emlen and Oring 1977, Seiki et al. 2005).

C. belfragei mean guarding and copulation durations were of significantly longer duration than those recorded for *C. formosa* (Table 3), suggesting that selection for longer pairings is stronger in *C. belfragei*. Due to the fact that in both species there were no significant differences in the extent of harassment of mating males by non-mating males, there was no apparent large male advantage in contests for females, and the OSR was not male-biased, the longer mating behavior durations in *C. belfragei* may be due to ecological factors. These factors may include a greater risk of predation while amplexed experienced by *C. formosa* compared to *C. belfragei* due to the lack of vegetation for cover in the *C. formosa* mating areas and the inability to fly while amplexed. Also, in *C. formosa*, the benefits to males from prolonged amplexus may be reduced due to a lower density of males in the mating area and also due to the possibility that *C. formosa* oviposition sites are outside of the mating area, which may reduce the availability of

mated females. However, more research needs to be performed to substantiate these points

Experiment 1: Mating Constraints (1 Male: 1 Female)

In the *C. belfragei* mating constraints and male choice experiments males copulated significantly longer with larger females (Figs 1 and 3; Table 1). This is likely due to selection for males that can assess female size and increase copulation duration for larger females. This may allow males to invest more energy or sperm in a larger female with higher fecundity. There were no other significant correlations of male or female size and guarding or copulation duration in the *C. belfragei* mating constraints and male choice experiments. This suggests that male size does not significantly influence these mating behaviors when no other males are present in the mating area.

In the *C. formosa* mating constraints experiment there was a significant positive correlation between female size and guarding duration (Fig 2; Table 2). This may be due to males increasing the amount of time spent guarding large females because of the higher potential fecundity of larger females. Results from the *C. formosa* male-male competition experiment indicated that females mate more than once without ovipositing, thus the longer guarding duration of larger females may be a strategy to protect the ejaculate from displacement by rival males. The mating constraints experiment is important because the results show the mating behaviors that occur when the mating pair is not affected by a non-mating male or by another female in the mating chamber.

Experiment 2: Male Choice (1 Male: 2 Females)

In both species there was no significant difference in the size of female that males chose to mate with in the male choice experiments, indicating that male choice of large females is unlikely in these species. Male *C. belfragei* did mate with ten large females and only five small females in the male choice experiment. However, the fact that seven *C. belfragei* males in this experiment mated with both the large and the small female, and the small sample size of this experiment reduced the power to obtain significant male choice correlations.

Female size was significantly positively associated with copulation duration in the male choice experiment with *C. belfragei*, indicating again that males may assess female size only when in physical contact with females (Fig 3). It is possible that males use tactile cues to determine female size, as has been shown in many studies of arthropods (Bondurianski 2001). This would explain the lack of male choice of large females, and the correlation of female size and duration of copulation in *C. belfragei* (Table 1). This is also supported by the finding that when males mated with both females, they did not mate with large females first more often than small females. Male size did not correlate with any of the mating behaviors evaluated in *C. belfragei* male choice trials, indicating that male *C. belfragei* mating durations were not significantly different for small and large males when two females were in the mating chamber.

Male *C. formosa* did not choose large females over small females, and female size did not correlate significantly with mating behavior durations, but male size was negatively associated with copulation duration (Fig 4; Table 2). This result was

unexpected, but may be due to smaller males investing more sperm in females regardless of female size. However, smaller males may be more vulnerable to takeover by other males during mate guarding, and extending the copulating phase may be a strategy to delay the time at which small males are more susceptible to being dislodged. It is also possible that small males are sperm deficient and may require more time to transfer sperm and accessory gland secretions, or smaller males may have slower rates of sperm displacement, which has been found in dungflies (Parker and Simmons 1994). The fact that this correlation did not occur in the mating constraints or male-male competition experiments suggests that the extra female in the mating area likely influenced this relationship.

Experiment 3: Female Choice

There was no significant difference between the amount of time a female *C. belfragei* or *C. formosa* spent near the small male compared to time spent near the large male, suggesting that females may not assess males based on visual or olfactory cues. It is also possible that females use other means of discriminating among males. In a study of the mating behavior of two *Pseudoxychilla* tiger beetles, Rodriguez (1998) found that females removed spermatophores after copulation in 36% of the 28 copulations recorded. If female *C. formosa* and *C. belfragei* can determine male genetic quality through courtship cues or other factors, it is possible that these females could use a post-mating strategy such as spermatophore rejection to greatly influence selection of male size and male mating behaviors such as mate guarding.

Experiment 4: Male-Male Competition (2 Males: 1 Female)

In the *C. belfragei* male-male competition trials there was a significant difference between the 1:1:1 mating frequency expected if large males have no mating advantage, with 13 of the 17 trials resulting in both males mating. However, *C. formosa* did not differ from the expected 1:1:1 mating frequency, with 14 of the 33 trials resulting in both males mating. This indicates that females do mate more than once without ovipositing, and suggests that sperm competition and male competition for females occurs in these species. The hypothesis that male competition is high in these species is also supported by the large number of attacks and removals of mating males, although neither small nor large males differed significantly in the number of attacks or success in removing mating males.

Contrary to the results of the mating constraints and male choice experiments, there were no significant correlations of female or male size and mating behavior durations (Table 1, Table 2). This effect of an extra male on the mating characteristics is expected in systems with a high degree of male-male competition. In mating systems with intense male competition for mates, selection will favor males that will mate with any female regardless of size (Crespi 1989). This could explain the observed lack of male choice of large females in these species. Although there was not a significant difference in the ability of large or small males to displace mating males, and mating male size relative to mating female size did not affect probability of mating male displacement, male competition is expected to reduce the ability of the mating male to maintain a mate, due to attacks and removals by non-mating males.

Due to the fact that both males mated in 13 of the 17 *C. belfragei* and 14 of the 30 *C. formosa* male-male competition trials, the mean size of the large and small male, and the means of mating behavior durations were used in the correlation tests. To further investigate the data within the trials in which both males mated, I used Wilcoxon signed-rank tests to look for differences in mating behaviors between the large and small males. None of the tests found significant differences between large and small male mating behavior durations in either species. This suggests that using the mean of male size and mean guarding and copulation durations should allow evaluation of the effect of male competition on mating characteristics. For the 14 *C. formosa* trials in which both males mated, the size difference between large and small males had no effect on any of the mating behaviors evaluated. For the 13 *C. belfragei* trials in which both males mated, the size difference between the large and small male was positively correlated with guarding duration (Fig 5). This would appear to be caused by males with a large size advantage monopolizing the females without disruption by the smaller males. However, there was a positive correlation between male size difference and the number of attacks or bouts on mating males (Fig 6), although the number of removals of mating males due to these bouts did not correlate significantly with the male size difference. This shows that in *C. belfragei* when there was a greater size difference between males, the guarding duration was greater and there were more attacks on mating males, but the likelihood of disruption was not significantly greater when male size difference was high. This suggests that when the large male was much larger than the small male, attacks on the mating male were more frequent, but did not reduce guarding duration as much as when the male size difference was small.

Body Size Correlations

Male *C. belfragei* copulated longer with larger females in both the mating constraints and the male choice experiments, which is likely due to increased male benefits when mating with larger females (Table 1). Even though male *C. belfragei* did not guard larger females longer in these experiments, increasing copulation time may be a means of monopolizing larger females. In the *C. formosa* mating constraints experiments guarding duration was significantly longer when males were paired with larger females (Table 2). This suggests that male *C. belfragei* and *C. formosa* may exhibit “cryptic” male choice by adjusting guarding and copulation duration to allocate the optimal amount of energy in a particular female. Larger females are likely to be more fecund and guarding and copulating longer may prevent females from remating before oviposition. Also, smaller male *C. formosa* copulated significantly longer in the male choice experiment. Smaller males may have more difficulty searching for and securing a mate than large males, which may favor small males that invest more in females. However, the pattern may reflect differences in the durations of sperm removal and transfer if smaller males are sperm deficient or have a slower rate of sperm displacement.

I observed females of both species apparently ovipositing eggs in the substrate with guarding males attached. Also, the male-male contests and male defensive reactions documented suggest that a high level of competition for mates occurs in these species. In mating observations males of both species performed shallow pumps with partial withdrawal of the aedagus in Phase 2 of mating with females. During this stage a wet substance was visible on the aedagus, possibly a previous male’s ejaculate. These observations indicate that male competition may be high and also that sperm precedence

is likely a characteristic of these species. These observations, and the fact that the females of both species in the male-male competition experiment often mated with both males, indicates that sperm competition is high in these species. This means that we can expect selection for males that can adjust mating behaviors to prevent remating of their partners and thus increase the likelihood of paternity. As with other time investment strategies, mating males should only remain with a female when the rate of gain due to guarding exceeds that due to withdrawal for further searching (Yamamura 1986).

The mean SOPm was slightly greater than 0.5 for both species in the male choice trials, indicating that the total time spent amplexed was approximately equal for both small and large females in this experiment (Fig 7). Similarly, the mean SOM by males in the male-male competition experiment was approximately equal for both the large and small males (Fig 7). Also, there was no significant difference in the probability of a large versus a small male mating first in the *C. belfragei* and *C. formosa* male-male competition trials. These findings further support the suggestion that although male competition is high in these species, there is not an apparent large male mating advantage.

Effects of the Social Environment: Intra and Interspecific comparisons

Intraspecific

There were no significant differences in *C. belfragei* guarding or copulation duration due to the type of experiment (Fig 8). This indicates that in *C. belfragei* these mating behaviors are not significantly affected by the changes in the sex ratio or social environment found in these three experiments. Conversely, all pairwise comparisons of

C. formosa guarding and copulation durations were significant across the experiments (Fig 9). In the mating constraints experiment, *C. formosa* male guarding and copulation durations were of significantly longer duration than in the male choice and male-male competition experiments. These mating behaviors were also significantly longer in the *C. formosa* male-male competition experiment than in the male choice experiment. These results indicate first that there is a significant disruptive effect in *C. formosa* from adding a third individual in the mating area. Second, this shows that in *C. formosa* male-male competition results in longer pairing durations than when the extra individual in the mating area is a female. This may result from males spending more time monopolizing a given female when another nearby male may remove his sperm before oviposition occurs. This also indicated that *C. formosa* males in the male choice experiment may be capable of determining that another female in the mating area does not pose such a threat. Third, this evidence shows that, compared to *C. belfragei*, male *C. formosa* are able to adjust mating behaviors more readily based on cues from the social environment.

Interspecific

Although no significant differences were detected between *C. belfragei* mating behavior durations across the three experiments, *C. belfragei* guarding and copulation durations were significantly longer than *C. formosa* behaviors in all three experiments (Table 1). This suggests that selection for longer pairing durations is stronger in *C. belfragei* than in *C. formosa*. Since the operational sex ratio was not male biased in either species, there were no significant differences in the extent of harassment of mating males by non-mating males between the species, and there was no apparent large male advantage in contests for females in either species, the longer mating behavior durations

in *C. belfragei* may be due to ecological factors. Shivashankar and Pearson (1994) compared mate guarding characteristics among five *Cicindela* tiger beetle species in India. The extent of male harassment during amplexus (attacks/minute of amplexus) did not explain the observed differences in mate guarding durations across the five species. However, mate guarding duration was significantly longer for species in which the female remained in the mating area longer after amplexus. These authors determined that in two of the species, females oviposited on abandoned termite mounds adjacent to the mating area. Males were absent in the ovipositing area, and the species in which females left the mating area soon after mating to oviposit elsewhere had significantly shorter mate guarding durations than the species in which the female remained in the mating area to oviposit (Shivashankar and Pearson 1994).

The significantly shorter amplexus durations displayed by *C. formosa* compared to *C. belfragei* may be affected by the duration of the female's stay in the mating area after amplexus. I observed *C. belfragei* females ovipositing in the mating area, and *C. belfragei* were always observed near the site where most mating pairs were collected. However, on many occasions *C. formosa* individuals were observed flying away from the mating area to adjacent areas with mounds of upturned sand, apparently from excavation by rodents. No mating was observed in these areas, which supports the idea that female *C. formosa* may leave the mating area soon after amplexus to oviposit in areas where males may be absent. Also, *C. formosa* are agile fliers but cannot fly while in amplexus, indicating that mating *C. formosa* may be much more vulnerable to predation while amplexed. *C. belfragei* do not fly and may not experience a significantly greater risk of predation while amplexed. The risk of predation to *C. formosa* while amplexed may also

be greater than *C. belfragei* due to the fact that *C. belfragei* mated in areas with dark soil and moderate vegetation cover, but *C. formosa* mated in open patches of light colored sand with little or no vegetation. Also, *C. belfragei* are a dull black color, while *C. formosa* are iridescent orange and red. These observations suggest that mating *C. formosa* may be more exposed to visually oriented predators during mating. A study of anti-predator mechanisms in adult tiger beetles (*Cicindela* spp.) revealed that robber flies, lizards, and birds were the most common threats to tiger beetles (Pearson 1985). In addition, although the operational sex ratio of both species did not differ significantly from 1:1, an estimate of the density of *C. belfragei* and *C. formosa* based on the number of individuals collected per minute of searching indicated that *C. belfragei* is likely to be more abundant in the mating area. This also increases potential benefits of mate guarding to *C. belfragei* males due to a higher probability of male competition for mates. Although *C. formosa* is more difficult to catch, only two or three attempted captures failed per collection day, indicating that the density estimate based on the number of beetles captured per minute searching is a reasonable estimate of the abundance of beetles in the field. The potentially longer stay in the mating area of mated *C. belfragei* females, the lower potential risk of predation of *C. belfragei* while in amplexus, and the greater density of *C. belfragei* in the mating area may increase the benefits of mate guarding for *C. belfragei* males.

Conclusions

The results of this study follow that of prior tests of sexual selection hypotheses for mate guarding. Intraspecific variability in mating behaviors may be correlated with one or more of the conditions that affect interspecific differences in mating behaviors.

Evaluating which conditions favor the evolution of behavioral flexibility in mating behaviors can help to reveal what processes may lead to intraspecific variation in mating behavior. It is important to recognize general patterns of how various social and ecological conditions may affect the evolution of different mating behaviors, because shared patterns suggest common causality. Further, documenting these patterns and investigating their possible causes can help shed light on fundamental questions about biodiversity in general, such as what factors promote diversification and speciation.

The results of this study indicate that mating behavior durations in the genus *Cicindela* are likely affected by the social environment, but may not be affected by the extent of mating male harassment by non-mating males or by the outcome of male contests for females. Instead this study supports the findings of a previous evaluation of *Cicindela* mating behavior by Shivashankar and Pearson (1994), that ecological factors may determine the costs and benefits of a particular mating strategy.

Future Studies

Future studies of tiger beetle mating system characteristics and sexual selection should include a thorough examination of how ecological and biological factors may affect the costs and benefits of particular mating behaviors. This can be accomplished by first observing mating females in the field to determine the amount of time spent in the mating area after termination of amplexus. This will require abstaining from collecting individuals in the field to allow sufficient field observations of the complete mating sequence and the behaviors of males and females after mating. Careful observation of females after mating may reveal whether females reject spermatophores, which could

affect selection of male traits. Second, locating the larvae of each species would help shed light on the potential for male competition, because the location of the larvae indicates the female oviposition sites, which may affect the duration of female stay in the mating area after mating and the availability of mated females. Third, if females oviposit outside the mating area, then it is critical to determine the presence or absence of males at the oviposition sites, which could affect the benefits of mate guarding. For instance, if males are absent in the oviposition areas and females move from the mating area to the oviposition area after amplexus, the benefits of mate-guarding for males could be reduced. Fourth, the time required for a mating pair to split up and run or fly away from a threat should also be evaluated. A significant difference in the reaction time of mating pairs between species could also affect the optimal duration of amplexus in a given species. Also, experiments should be performed to estimate whether the risk of predation during amplexus is greater for males or females, because intersexual differences in the risk of predation could affect mating strategies. Lastly, the characteristics of sperm production and female insemination should be evaluated.

Table 1: Results of *C. belfragei* Pearson's correlation tests between guarding and copulation durations and male and female size in the three mating experiments.

One-tailed tests were used for female size tests as longer mating behavior durations are expected with larger females. Two-tailed tests were used for male size comparisons.

Exp. 1: Mating constraints

Test	r	t	p
female size vs guarding	0.11	0.63	0.26
female size vs copulation	0.50	3.02	0.0026
male size vs guarding	-0.09	-0.50	0.62
male size vs copulation	0.19	1.07	0.29

Exp 2: Male choice

female size vs guarding	0.23	1.04	0.156
female size vs copulation	0.42	2.06	0.0265
male size vs guarding	0.065	0.29	0.77
male size vs copulation	-0.015	-0.066	0.95

(Table 1 - Continued)**Exp. 3: Male-male Competition**

Test	r	t	p
female size vs guarding	-0.23	-0.9	0.80
female size vs copulation	0.027	0.11	0.54
male size vs guarding	-0.42	-1.79	0.094
male size vs copulation	-0.44	-1.9	0.076

Table 2: Results of *C. formosa* Pearson's correlation tests between guarding and copulation durations and male and female size in the three mating experiments.

One-tailed tests were used for female size tests as longer mating behavior durations are expected with larger females. Two-tailed tests were used for male size comparisons.

Exp. 1: Mating constraints

Test	r	t	p
female size vs guarding	0.29	1.69	0.05
female size vs copulation	-0.02	-0.13	0.55
male size vs guarding	-0.12	-0.60	0.55
male size vs copulation	-0.08	-0.42	0.68

Exp. 2: Male choice

female size vs guarding	0.153	0.83	0.22
female size vs copulation	0.20	1.08	0.15
male size vs guarding	0.075	0.41	0.69
male size vs copulation	-0.41	-2.42	0.022

(Table 2 – Continued)**Exp. 3: Male-male competition**

Test	r	t	p
female size vs guarding	0.16	0.88	0.19
female size vs copulation	0.13	0.72	0.24
male size vs guarding	-0.11	-0.64	0.52
male size vs copulation	-0.16	-0.93	0.35

Table 3: Mean guarding and copulation durations of *C. belfragei* and *C. formosa* males in the three mating experiments.

Mean ($\pm$ s.e.m.) guarding, copulation, and total amplexus durations (min) for *C. belfragei* and *C. formosa* in each of the three experiments (mcon = single male and female; mchoice = male with a large and small female; mmcomp = two males and a single female). All pairwise comparisons of guarding (g) and copulation (c) durations indicated that these mating behaviors were of significantly longer duration in *C. belfragei* than in *C. formosa* experiments.

Species	Experiment	<i>n</i>	mean g	mean c	mean ta
<i>C. belfragei</i>	mcon	30	28.3 ± 5.0	23.0 ± 2.7	52.3 ± 7.1
	mchoice	22	25.9 ± 10	21.8 ± 2.6	47.7 ± 11.4
	mmcomp	17	40.82 ± 8.7	24.2 ± 3.6	65.82 ± 11.3
<i>C. formosa</i>	mcon	33	18.2 ± 3.3	9.3 ± 1.5	26.9 ± 3.8
	mchoice	31	2.2 ± 0.5	3.87 ± 0.5	6.0 ± 0.8
	mmcomp	33	7.0 ± 1.8	5.5 ± 1.0	12.6 ± 2.0

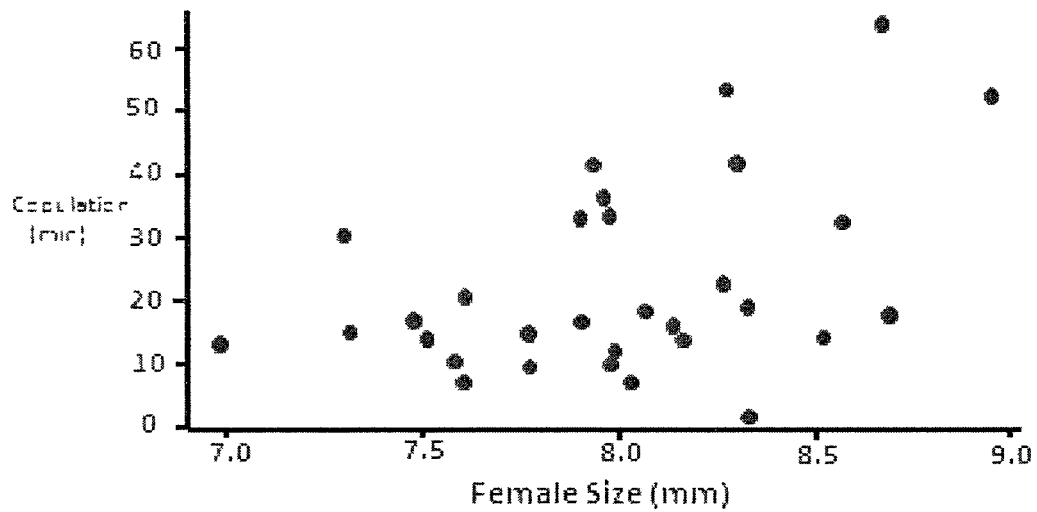


Figure 1: Positive correlation between female size and copulation duration in the *C. belfragei* male choice experiment.

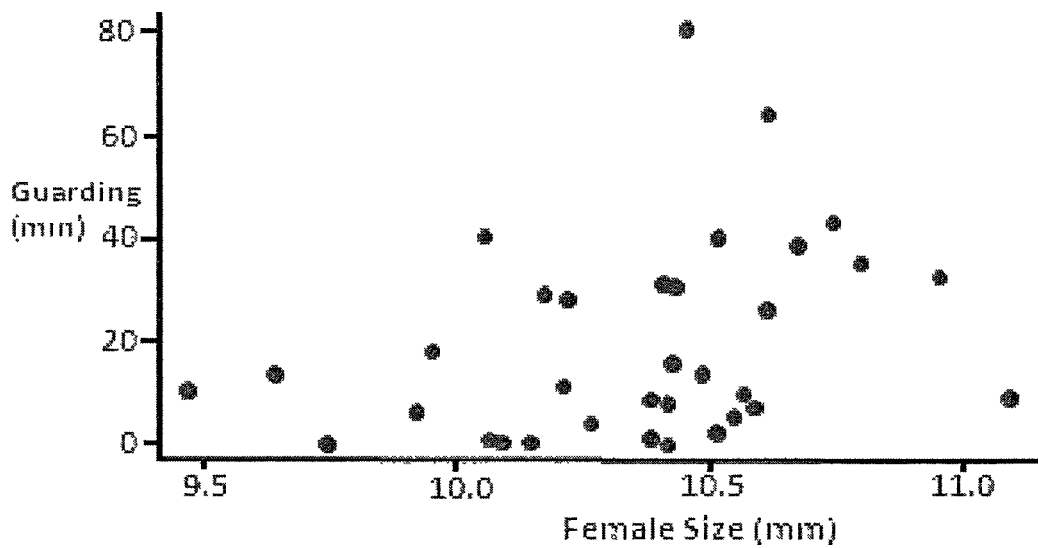


Figure 2: Positive correlation between female size and guarding duration in the *C. formosa* mating constraints experiment.

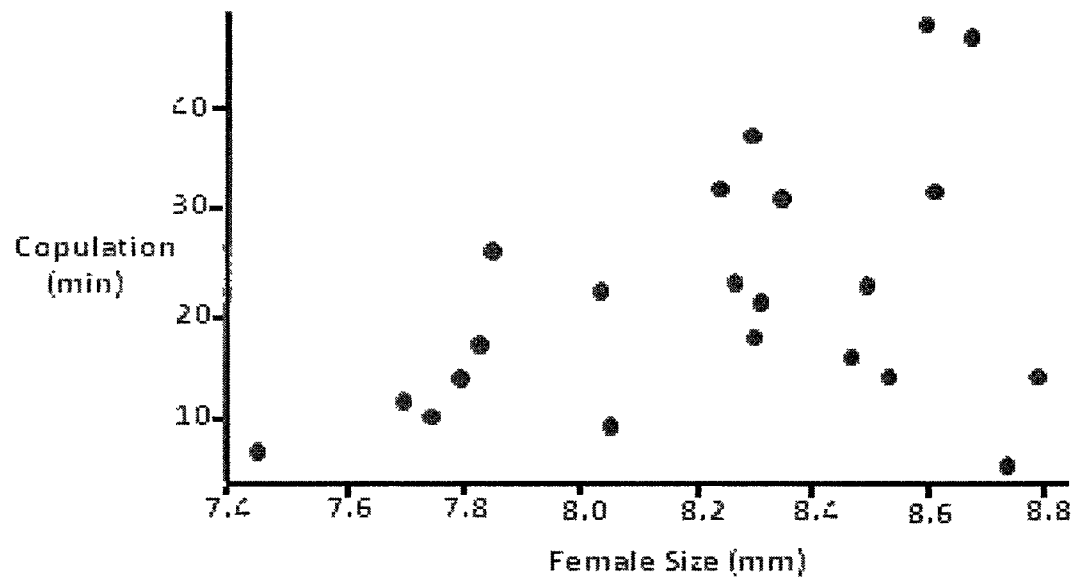


Figure 3: Positive correlation between female size and copulation duration in the *C. belfragei* male choice experiment.

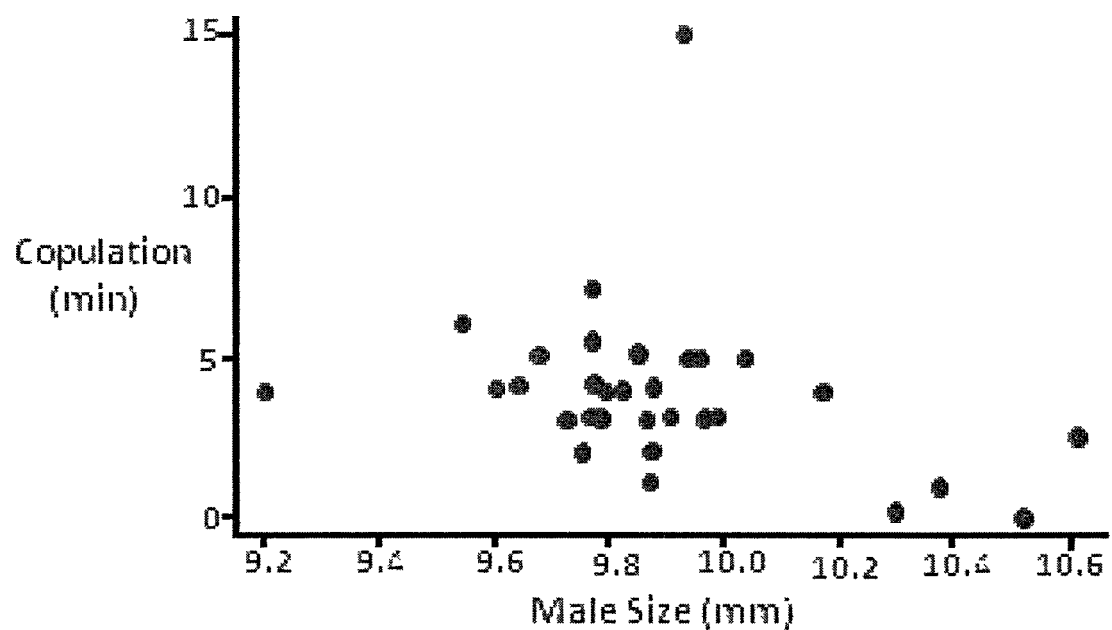


Figure 4: Negative correlation between male size and copulation duration in the *C. formosa* male choice experiment.

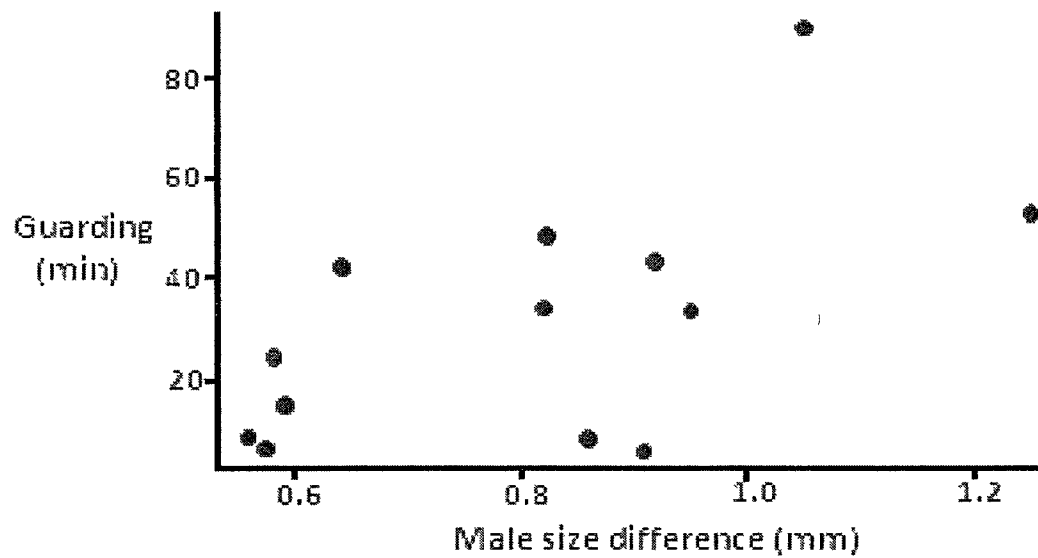


Figure 5: Positive correlation between male size difference and guarding duration in the *C. belfragei* male-male competition experiment.

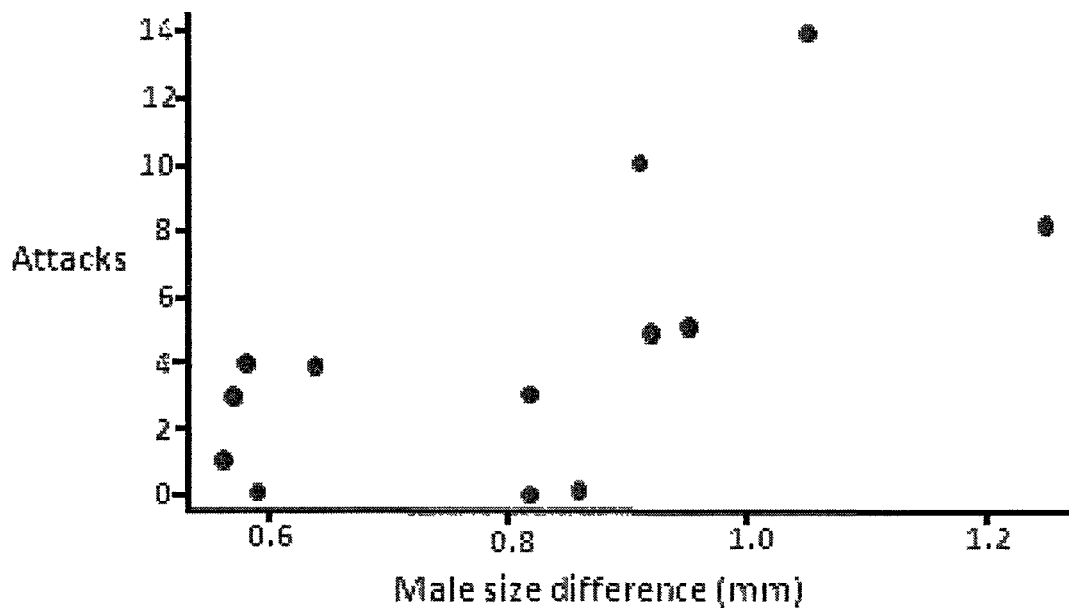


Figure 6: Positive correlation between male size difference and the number of attacks on mating males in the *C. belfragei* male-male competition experiment.

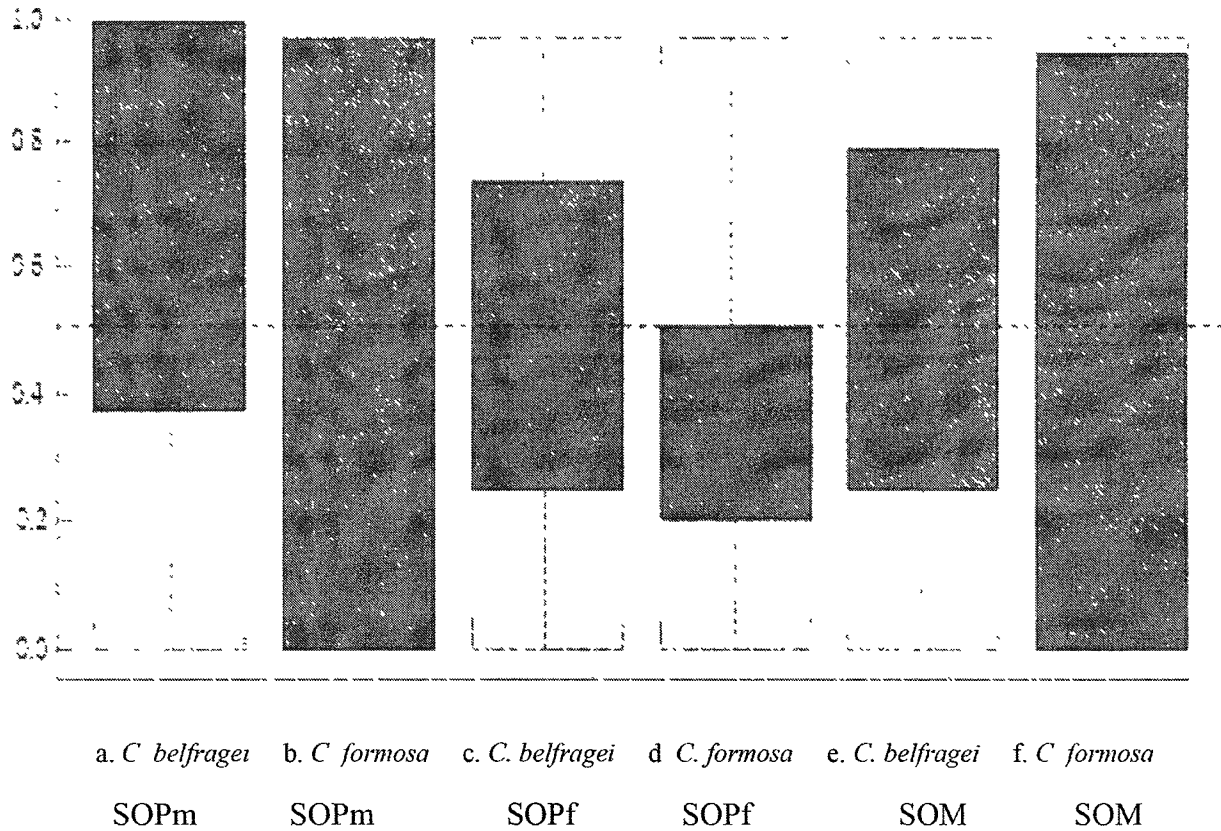


Figure 7: *C. belfragei* and *C. formosa* boxplots; male strength of preference (SOPm) for large females in the male choice experiment, female strength of preference (SOPf) for large males in the female choice experiment, and strength of mating (SOM) by large males in the male-male competition experiment.

Boxplots of strength of preference for : (a) *C. belfragei* male choice experiment (N = 22), (b) *C. formosa* male choice experiment (N = 31), (c) *C. belfragei* female choice experiment (N = 17), (d) *C. formosa* female choice experiment (N = 39), (e) *C. belfragei* male-male competition experiment (N = 17), and (f) *C. formosa* male-male competition experiment (N = 33).

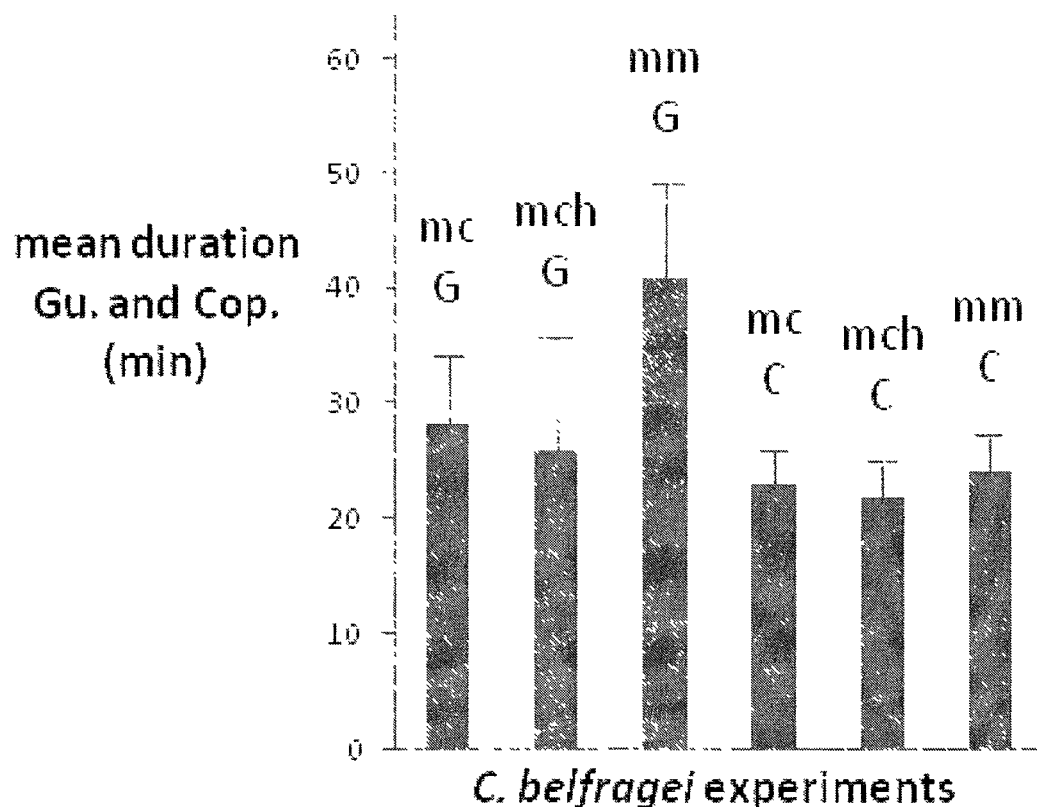


Figure 8: Bar chart of mean guarding and copulation durations in the *C. belfragei* mating experiments.

Two-tailed Welch modified two sample *T*-tests were used to perform all pairwise comparisons of mean guarding (G) and copulation (C) durations (min) between the three experiments (mc = single male and female; mch = male with a large and small female; mm = two males and a single female; s.e.m. shown above each bar) for *C. belfragei*. No significant differences were found (NS) between the mating behavior durations of the three experiments ($p > 0.2$).

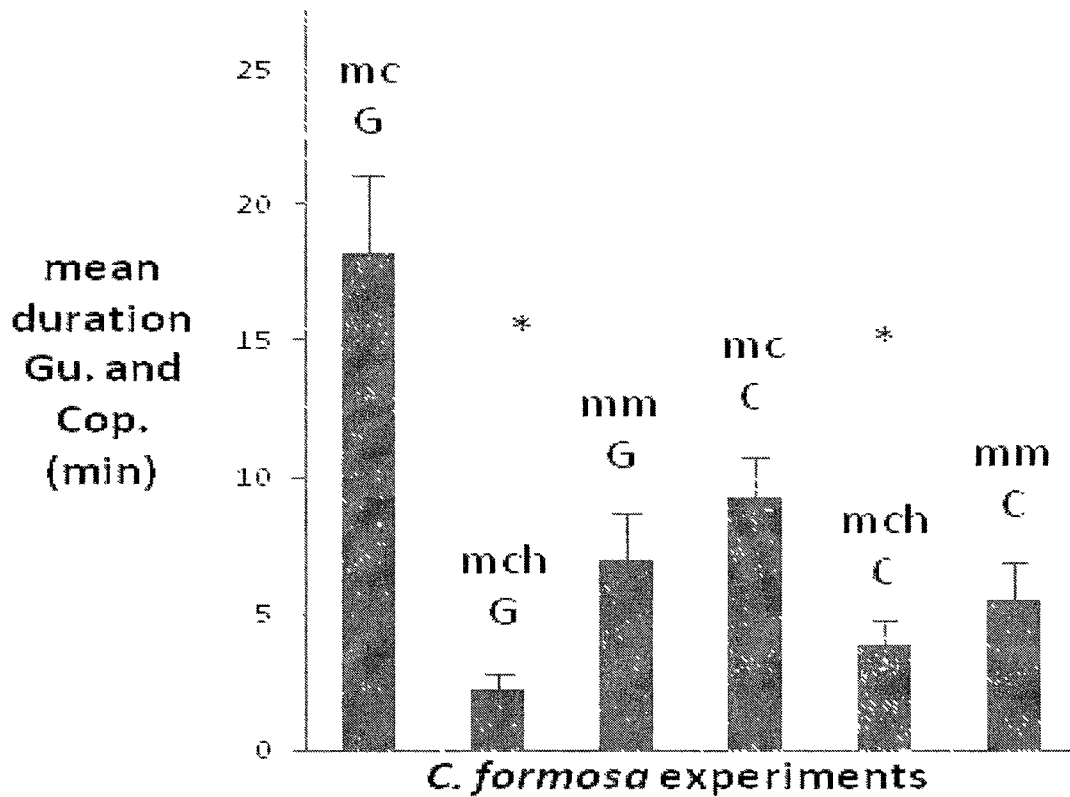


Figure 9: Bar chart of mean guarding and copulation durations in the *C. formosa* mating experiments.

Two-tailed Welch modified two sample *T*-tests were used to compare mean guarding (G) and copulation (C) durations (min) between the three experiments (mc = single male and female; mch = male with a large and small female, mm = two males and a single female; s.e.m. shown above each bar) for *C. formosa*. All tests found significant differences (* $p < 0.05$).

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CHAPTER II: SIZE ASSORTATIVE MATING AND SEXUAL SIZE DIMORPHISM

INTRODUCTION

According to Darwin (1871), sexual selection arises from differences in reproductive success caused by competition over mates. This competition includes not only contests for mates, but also mate choice due to the fact that choice of a particular mate by an individual makes the resource (mates) harder to acquire for others. Sexual selection on a trait can therefore be interpreted as differences in reproductive success, caused by competition over mates, and related to the expression of the trait (Andersson 1994). Males, females, or both sexes may prefer larger mates because they benefit reproductively and are differentially capable of exercising choice (Ridley 1983, Rowe and Arnqvist 1996). Males are expected to benefit from choosing large females because egg production increases with body size in many species (Andersson 1994), especially in insects (Thornhill 1976, Preziosi et al. 1996, Logan et al. 2001). The benefits to females from choosing large males is not as straight forward, but females may benefit because large males are more fertile (Howard et al. 1998, McLain 1998). If small males are sperm limited, this could result in selection for female choice of larger males.

Alternatively, if larger males are preferred then the offspring of females who mate with larger males may have larger and more successful male offspring.

To evaluate sexual selection within any species or population one must investigate the relationship between variation in behaviors and variation in fitness. This can be done by testing whether there is a correlation within or across species of certain traits and particular mating system characteristics or ecological factors (Thornhill and Alcock 1983). When comparing unrelated species, the underlying premise is that similar environmental conditions should result in the evolution of analogous traits. On the other hand, divergent selective pressures acting on closely related species should lead to the evolution of distinctive mating system characteristics within each species. Population characteristics such as size assortative mating and sexual size dimorphism should correlate with mating behaviors or ecological conditions in mating systems with prolonged post-copulatory associations, due to selection for optimal mating strategies when individuals experience high costs associated with mating (Kraus and Lederhouse 1983, Juliano 1992, Harrari et al. 1999, Yoshitakke et al. 2004).

Size Assortative Mating

Size assortative mating, or homogamy, defined as a positive correlation between the sizes of mates in populations, is one of the most common mating patterns found in nature (Ridley 1983, Crespi 1989, Arnqvist et al. 1996). Assortative mating has been of interest to evolutionary biologists because it typically promotes the maintenance of genetic variation within populations (Ridley 1983, Partridge 1983, Arnqvist et al. 1996, Parker and Partridge 1998). When individuals compete for mates, size assortative mating

can result if large size confers a competitive advantage (Johnson 1982, Rowe and Arnqvist 1996). Competition for mates as a cause of assortative mating has been documented almost exclusively among males (Crespi 1989), probably due to the fact that females are more often the limiting resource (Trivers 1972).

Crespi (1989) conducted a comparative analysis on the causes of assortative mating in arthropods and concluded that assortative mating can be caused by many factors. Crespi (1989) proposed three processes that can explain assortative mating: 1) mate choice, when large males and/or large females choose to mate with large mates, often due to reproductive benefits; 2) mate availability, when large individuals are differentially available for mating due to size differential variation in mating probability that is not due to mate choice; 3) mating constraints, when relative size differences between males and females cause physical difficulties with courtship, pairing, mating, or mate guarding. Physical difficulties may arise if very small males have difficulty mating with very large females, or vice versa, due to mechanical incompatibilities of genitalia or coupling structures.

There are two forms of assortative mating, the 'true' form and the 'apparent' form (Crespi 1989, Arnqvist et al. 1996). True assortative mating occurs when there is a linear relationship between the sizes of mating males and females, where observations are symmetrically distributed around the regression line. Apparent assortative mating occurs when a positive regression results from increased or decreased variance in male size with increased female size. This apparent form will only occur when the strength of any large or small male mating advantage is related to the size of the mate (Crespi 1989, Arnqvist et al. 1996). This type of relative male advantage will occur if large males are able to

mate with all females, but small males are restricted to mate primarily with small females. This can also occur if small males mate with all females, but large males only mate with large females. Distinguishing between these forms of assortative mating is important, because apparent assortment will not promote the maintenance of genetic variation as readily as true assortative mating (Crow and Felsenstein 1968, Partridge 1983, Rowe and Arnqvist 1996). In populations with apparent assortative mating, genetic variance will be diluted by random mating at either large male or small male size extremes.

Sexual Size Dimorphism

In mating systems with strong sexual selection of mate size, sexual size dimorphism (SSD) is likely to accompany assortative mating (Shine 1989). SSD is a widespread phenomenon among animals, and occurs when one sex has a larger body size than the other sex. Sexual selection is the most commonly proposed cause of SSD (Juliano 1992), but other factors may be involved, such as intersexual resource partitioning (Shine 1989), intersexual differences in growth rates (Kozłowski 1989), or mechanical constraints resulting from one sex carrying the other sex during mating (Wicklund and Forsberg 1991). The sexual selection hypotheses for SSD predicts that differences in the relationship between body size and reproductive success between males and females results in selection for different adult body sizes in the two sexes (Shine 1989).

Females are larger than males in many insects, and increased fecundity of large females (Thornhill 1976, Preziosi et al. 1996, Logan et al. 2001), which has been found in

many taxa, may cause male choice of large females (Andersson 1994). Male-male competition has been implicated as the cause of SSD when males are larger than females, due to increased reproductive success through direct competition for females (Andersson 1994). However, direct male-male competition may still occur in sexually dimorphic species where females are larger than males (Kraus and Lederhouse 1983, Brown 1990, Brown and Stanford 1992, Shivashankar and Pearson 1994, Arnqvist et al. 1996), but in these species larger female size is only expected to occur when selection for large female size is stronger than selection for large male size (Arnqvist and Rowe 2005).

Mate Guarding

Many species of arthropods have a mating system in which males remain mounted on the female for extended periods of time after sperm transfer (Kraus and Lederhouse 1983, Brown 1990, Carroll 1993, Shivashankar and Pearson 1994, Fielding and Knisley 1995, Arnqvist et al. 1996), likely due to sperm competition (Parker 1970). In these species the time a male spends attached to females after copulation is often considered mate guarding (Alcock 1994). Studies of arthropods with this type of mating system have implicated mate choice and male-male competition as proximate factors influencing assortative mating by size (Kraus and Lederhouse 1983, Brown 1990, Brown and Stanford 1992, Harari et al. 1999) and sexual size dimorphism (Juliano 1992, Yoshitake 2004). The cost to males incurred by long periods of time invested on each female may favor males that prefer to mate with larger females (Andersson 1994). Male choice of large females and male-male competition in these systems may cause positive size assortative mating when large males monopolize large females, leaving small males

to mate with small females (Crespi 1989). Female-biased SSD is also expected to be common in taxa with this type of mating system due to male choice of large females.

Simmons (2001) suggested that whenever post copulatory associations occur, selection will favor adaptations that improve the efficiency of the association. Morphological traits such as male mandibles and female coupling sulci that function as holdfast devices during takeover attempts by rival males may become well developed in species with high levels of sperm competition. This may benefit the female by allowing her to lay more eggs without constantly mating, especially in species where the male guards the female during oviposition (Pearson 1988). Also, male dwarfism may function in reducing the costs incurred by females from carrying males around during amplexus (Wicklund and Forsberg 1991). In most species of tiger beetles, males are significantly smaller than females (Pearson 1988). Female-biased sexual size dimorphism may have evolved due to selection for smaller males when female energy expenditures due to mating are high.

Kraus and Lederhouse (1983) investigated mate guarding behavior in a population of tiger beetles, *Cicindela marutha*, in southeastern Arizona. They determined that copulation only accounted for 2.3% of the time amplexed pairs were observed (Kraus and Lederhouse 1983). Males that maintained amplexus after copulation were attacked by single males which attempted to displace them (Kraus and Lederhouse 1983). The authors suggested that the post-copulatory amplexus observed was likely a mate guarding strategy that could result in an increase in the number of eggs fertilized by their sperm.

The demonstration that post-copulatory amplexus results in males reducing remating of their partners and increasing egg fertilization by their sperm is evidence that the behavior involves sperm competition by males (Alcock 1994). However, many factors may affect the benefits derived from post-copulatory amplexus among species. To determine which factors influence differences in mate guarding duration one must investigate the relationship of the intensity of sexual selection on males and the duration of mate guarding (Alcock 1994). Many factors can influence the optimal amount of energy and risk a male should invest in a particular female (Yamamura 1986). The duration of mate guarding by males that maximizes reproductive success may be affected by the degree of male competition, availability of females, predation pressures, and thermoregulatory capabilities (Yamamura 1986, Carroll 1993, Alcock 1994, Pearson 1988). Schultz (1998) showed that enhanced thermoregulation is not a likely benefit derived from mate guarding among tiger beetles. Also, since tiger beetles in amplexus may experience a greater risk of predation, the extent of that risk may influence the optimal mate guarding duration among tiger beetle species.

Field observations were used to investigate mating system characteristics in two tiger beetle species, *Cicindela (Dromochorus) belfragei* and *Cicindela (Dromochorus) formosa*. Specifically, I focused on assortative mating and sexual size dimorphism. I also examined factors that may influence observed adult body size such as the availability of males and females of different sizes throughout the mating season and at different collecting sites.

Study System

Cicindela formosa occurs in the Lost Pines Region of central Texas, and *C. belfragei* occurs in the Edwards Plateau of central Texas. Assortative mating by size has been observed in several beetle families (Cantharidae: McLain 1982, Meloidae: Brown 1990 and Brown 1993, Curculionidae: Harari et al. 1999), but has only been documented in one species of tiger beetle, *C. marutha* (Kraus and Lederhouse 1983). However, the biology of tiger beetles suggests that assortative mating might also occur in other species within the Cicindelidae (Pearson 1988), and several lines of evidence suggest that assortative mating should be expected in *C. belfragei* and *C. formosa*. 1) There is substantial body size variation within these two tiger beetle species, which is necessary for size assortative mating. 2) Both species exhibit a prolonged post-copulatory mounting phase. 3) These species are both sexually dimorphic in body size, with females larger than males.

Objectives

Determining the factors that may generate assortative mating and SSD in taxa that exhibit mate guarding behavior requires two lines of investigation. First, the pattern of assortative mating in the field must be documented and, second, processes that lead to size biases in each component of mating must be identified through laboratory experiments (Rowe and Arnqvist 1996). In this study I combined these two levels of inquiry to evaluate the pattern of assortative mating and the processes that may influence assortative mating and SSD in *C. belfragei* and *C. formosa*, and asked the following questions:

1. Do *C. belfragei* and *C. formosa* exhibit assortative mating?

Field observations provide an estimate of assortative mating in the field, and also shed light on whether assortative mating is of the true or the apparent form (Crespi 1989).

2. Do *C. belfragei* and *C. formosa* exhibit SSD?

Field measurements were performed to examine the extent of SSD, which should reflect the strength of selection for large female or small male size in these species.

3. Does variation in mate availability contribute to assortative mating and SSD?

Observational data were used to determine whether factors such as spatial or temporal covariation in body size distributions affects observed mating patterns and SSD.

4. Are the mating behavior sequences of *C. belfragei* and *C. formosa* similar to other tiger beetles that have been examined?

If differences are detected they may have functional and biological significance. This information is critical for any comparative evaluation of mating behaviors between these species.

The analysis of field collected data provides an estimate of assortative mating in the field and allows assessment of the potential for non-sexual selection phenomena (mate availability) to influence assortative mating and SSD. The field experiments also reveal whether true or apparent assortative mating is occurring among these species.

MATERIALS AND METHODS

Study Sites

To evaluate assortative mating in *C. belfragei*, individuals were collected on private property in the Edwards Plateau near San Marcos, Texas. *Cicindela belfragei* were collected in open areas with sparse vegetation and relatively abundant soil at least once a week from May through early July 2006 and again in 2007, the period of mating activity for this species. To investigate the mating characteristics of *C. formosa*, I visited the Griffith League Ranch and Texas State's Welsh property in the Lost Pines near Bastrop, Texas approximately once a week from March through May 2006 and 2007. This is the period of mating activity for *C. formosa* (Schultz 1989).

Data Collected

Mating pairs in the field were collected, and the size (left elytron length) of each individual in the pair was documented. Also, the sizes of all beetles collected were used to determine mean male and female size for 2006 and 2007 in each species. Also, the data collected in the laboratory experiments performed in chapter I of this manuscript were used in this chapter to evaluate assortative mating and sexual size dimorphism.

Observational Experiment 1: Assortative Mating in the Field

To determine if assortative mating is occurring in the field I documented all mating pairs observed in 2006 and 2007 ($N = 31$ *C. belfragei*, $N = 5$ *C. formosa*). I used Pearson's product moment correlation tests for *C. belfragei* to test for correlation of mating male and female body sizes. No statistical tests were performed on the *C. formosa* mating pair data due to the small sample size of *C. formosa* mating pairs in the

field. The operational sex ratio across the collection dates was evaluated for bias in each species using χ^2 goodness-of-fit-tests. Two-tailed T -tests were used to determine if the mean size of mating *C. belfragei* males or females differed significantly from the mean size of non-mating males and females collected in the field. These non-mating individuals were the beetles collected and measured for use in the laboratory experiments. The test of the size of mating versus non-mating individuals was not performed for *C. formosa* due to the extremely small sample size in this study. Heteroscedasticity of mating pair sizes was measured to reveal whether assortative mating was true or apparent for these species (Crespi 1989). For instance, if large males only mate with large females, but small males mate with all females, this pattern would indicate that apparent assortative mating may be occurring rather than true assortative mating.

Observational Experiment 2: Sexual Size Dimorphism

To determine if female-biased sexual dimorphism is a characteristic of these species I compared the mean female size and mean male size in each species of all beetles collected in 2006 and separately in 2007 using two-tailed T -tests. I also computed the ratio of mean female to mean male size.

Observational Experiment 3: Mate Availability

To test the hypothesis that assortative mating occurs due to the availability of mates, I compared the sizes of males and females collected on different sampling dates for *C. belfragei* using a single factor ANOVA, with body size as the dependent variable and collection date as a factor. There was only one collection site for *C. belfragei*, so

tests of site effects on size distributions were not possible. However for *C. formosa* single factor ANOVAs were used to evaluate the effect of collection site and collection date on the size of males and females. If there was spatial or temporal covariation in body size distributions, then mate availability may influence SSD and field estimates of assortative mating due to size biases in the frequency of contact between size types.

Observational Experiment 4: Mating Behavior Sequence

I compared mating sequence behavior between *C. belfragei* and *C. formosa*, and I also compared the observed mating sequences with those previously described for tiger beetles. I based the description of the mating sequence behavior on the mating observations in laboratory experiment 1 to avoid the effects on mating behaviors due to two females in the mating chamber and male competition for a single female.

RESULTS

Observational Experiment 1: Assortative Mating in the Field

Mating male and female *C. belfragei* observed in the field did not pair according to size (Pearson's: $r = 0.035$, $df = 29$, $t = 0.189$, $p = 0.426$). However, the mean size of female *C. belfragei* observed mating in the field in 2006 were 4% larger than the mean size of all females collected in 2006 (T -test; $df = 30.4$, $t = 2.42$, $p = 0.011$). There was no significant difference between the size of mating and non-mating male *C. belfragei* in 2006 (T -test; $df = 24.4$, $t = -0.48$, $p = 0.63$), nor was there a significant difference between the size of mating and non-mating male *C. belfragei* in 2007 (T -test; $df = 24.4$, $t = 0.48$, $p = 0.31$), or mating and non-mating female *C. belfragei* in 2007 (T -test; $df = 30$, $t = -1.12$, $p = 0.87$). Only five mating pairs of *C. formosa* were observed and collected in

the field. Due to the small sample size and the resulting lack of power to detect a correlation of mating male and female body size, no statistical tests were performed.

There were more males than females of *C. belfragei* collected in 2006 (males = 75, females = 70) and 2007 (males = 79, females = 75), but the operational sex ratio was not male biased ($X^2 = 2.29$, $p > 0.1$). As in *C. belfragei* there were more male than female *C. formosa* collected in the field in 2006 (males = 64, females = 48) and in 2007 (males = 131, females = 112) but the operational sex ratio was not male biased ($X^2 = 1.48$, $p > 0.2$).

Observational Experiment 2: Sexual Size Dimorphism

The mean size of all female *C. belfragei* collected both in 2006 ($X = 7.89 \pm 0.06$ mm) and in 2007 ($x = 8.28 \pm 0.06$ mm) was significantly greater than the mean size of male *C. belfragei* collected in 2006 (7.31 ± 0.06 mm) and 2007 (7.73 ± 0.05 mm) (2006 *T*-test; $df = 86$, $t = 7.04$, $p = 0$; 2007 *T*-test; $df = 103$, $t = 7.51$, $p = 0$). In 2006 and in 2007 female *C. formosa* (2006: 10.24 ± 0.12 mm; 2007: 10.32 ± 0.04 mm) collected were larger than males collected in 2006 and 2007 (2006: 9.76 ± 0.05 mm; 2007: 9.81 ± 0.03 mm) (2006 *T*-test; $df = 107$, $t = 5.39$, $p = 0$; 2007 *T*-test; $df = 195$, $t = 11.1$, $p = 0$). The ratio of female to male mean size, an appropriate index of SSD, was 1.07 for *C. belfragei* and 1.05 for *C. formosa*.

Observational Experiment 3: Mate Availability

The mean size of male and female *C. belfragei* collected in 2006 (male mean = 7.31 ± 0.06 , female mean = 7.89 ± 0.06) and 2007 (male mean = 7.73 ± 0.05 , female mean = 8.28 ± 0.06) did not differ significantly between collection dates (ANOVA, $p >$

0.05). Also, the body size distributions of male and female *C. formosa* collected in the field on different dates and at different sites did not deviate from the mean size within each sex (ANOVAs; date: $p > 0.15$, site: $p > 0.25$)

Observational Experiment 4: Mating Behavior Sequence

The general mating sequence displayed by *C. belfragei* and *C. formosa* occurred in three stages (based on experiment 1: *C. belfragei* N = 30, *C. formosa* N = 33), similar to the three phase sequence described by Palmer (1976) for the tiger beetle species, *Pseudoxychila tarsus*. Phase 1 observed in all three species consisted of males mounting females and inserting the aedagus for about one minute. In *C. belfragei* and *C. formosa* instead of complete withdrawal during phase 2 described for *P. tarsus*, a partial withdrawal and slight pumping of the aedagus was observed. Similar to *P. tarsus*, in phase 3 male *C. belfragei* and *C. formosa* deeply inserted the aedagus. However, during phase 3 *C. formosa* males performed 8 to 14 deep thrusts of the aedagus, while males of the other species did not exhibit this behavior. Also, following phase 3 male *C. belfragei* maintained amplexus for a longer period of time than both *C. formosa* and *P. tarsus* (*C. belfragei* mean guarding = 28 minutes, *C. formosa* = 18 minutes, *P. tarsus* mean guarding = < 1 minute).

***Cicndela belfragei* and *C. formosa* Mating Behavior Characteristics**

The general mating sequence displayed by *C. belfragei* and *C. formosa* in the mating chamber in experiment 1 (single male and female in mating chamber; *C. belfragei* N = 30, *C. formosa* N = 33) began with the male following the female then mounting the female, usually within two minutes. Females often evaded males by running and jostling,

but most males mounted the female successfully on the first attempt. Once mounted, males locked mandibles into female coupling sulci on the mesepisternum. After mounting, males of both species inserted their aedagus completely for up to two minutes (phase 1), then withdrew to $\frac{1}{2}$ or $\frac{1}{4}$ insertion for approximately two minutes (phase 2). During this stage the male would pump the aedagus slightly and the aedagus often appeared wet, possibly with a previous male's ejaculate. The male would sometimes withdraw the aedagus completely for only a second or two during this stage of mating. Next the male inserted the aedagus $\frac{3}{4}$ of the way in, usually within 2 to 5 minutes of the initial mounting of the female (phase 3). In *C. belfragei*, the male pumped the aedagus slightly during this period, and sometimes twitched his front leg tips out to the side. In *C. formosa* males often performed 8-14 deep thrusts of the aedagus during phase 3, and also sometimes twitched their leg tips out to the side. In a previous study of mating behavior in tiger beetle, genus and species, s it was discovered that sperm transfer occurs in the third phase of mating (Freitag et al. 1980). This period of full insertion lasted an average of 14 ± 1.9 minutes in *C. belfragei*, and 3.5 ± 0.8 minutes in *C. formosa*, then the male withdrew the aedagus. The guarding stage lasted an average of 28 ± 5 minutes in *C. belfragei*, and 18 ± 3.3 minutes in *C. formosa*, then the pair split. During the guarding stage several females were observed scooping up soil with their mandibles, which is known to be part of the process of oviposition (Pearson 1988). Also, after withdrawal of the aedagus (end of phase 3 of mating), males would sometimes repeat phases 1-3. The pair split up after a mean of 52 ± 7.1 minutes of amplexus in *C. belfragei*, and 27 ± 3.8 minutes in *C. formosa*, usually without struggle by the female, but sometimes as a result of the female rocking back and forth. The female behavior of rocking back and forth was

often followed by a violent shaking reaction from the male, apparently an attempt to maintain amplexus. In field observations of both species amplexed females were observed vertically positioning their bodies and digging in the substrate with their ovipositors. Then the females seemed to flex the ovipositors, as if to release an egg into the hole. This occurred with the guarding male attached, and the process was repeated several times, with each hole spaced about 30 cm apart. Although the female may have been ejecting sperm, as spermatophore rejection has been documented in tiger beetles (Rodriguez 1998), the multiple holes dug by the female suggests egg deposition was occurring. This ovipositing behavior was also observed in the mating chamber during several *C. belfragei* mating trials.

Male-Male Contests

Non-mating males of *C. belfragei* and *C. formosa* in the male-male competition experiment most often attacked the mating male by ramming the rear of the mating pair with their mandibles. The mating male always reacted by kicking at the attacker with the rear and middle legs. This defensive reaction was displayed by the mating male even when the non-mating male was simply walking past the mating pair. On several occasions the non-mating male attacked from the front, sometimes locking mandibles into the female coupling sulci along with the mating male. On rare occasions, the non-mating male locked his mandibles onto the mating male's mesepisternum, and proceeded to attempt copulation with the mating male. This behavior was rare, but males may be able to lock their mandibles onto other males even though the coupling sulci are absent in male tiger beetles.

DISCUSSION

Cicindela belfragei and *C. formosa* did not pair according to body size in the field. This suggests that size assortative mating may not be a characteristic of these two species. However, in both species females were significantly larger than males, indicating that selection for large female size may be greater than selection for large male size.

Observational Experiment 1: Assortative Mating in the Field

I found that mating male and female *C. belfragei* collected in the field did not pair according to size. However, female *C. belfragei* observed mating in the field in 2006 were significantly larger than non-mating females in 2006. These results suggest that although male size may not predict mating success in the field, selection for larger females may operate in *C. belfragei*. This selection could result from male choice of large females, but this was not supported by the male choice experiments.

Due to the fact that only five mating pairs of *C. formosa* were observed and collected in the field, I did not perform a statistical test to evaluate the possibility of a correlation between mating male and female size. The *C. formosa* collection sites are spread out over a much larger area than the *C. belfragei* site, making mating observations in the field much less common. Also, the fact that *C. formosa* mean amplexus duration was significantly shorter than that of *C. belfragei* results in a lower probability of mating observations in the field. Due to the small sample size I cannot determine the likelihood of assortative mating in *C. formosa* from field observations.

Although assortative mating by size was not detected in field observations for *C. formosa* or *C. belfragei*, it is important to evaluate how the mating characteristics in this study may influence field estimates of assortative mating. In *C. belfragei*, mating constraint and male choice experiments males copulated longer with larger females, and in *C. formosa* mating constraint experiment, larger females were guarded longer, but there was no significant difference between the total amplexus durations (not reported here) of large versus small females in both species. This indicates that assortative mating estimates based on mating pairs observed in the field should reflect the true frequency of mating pair size combinations. In 2006, the mean size of female *C. belfragei* observed mating in the field was significantly larger than the mean size of all females collected. This suggests that, if assortative mating were detected in *C. belfragei*, it may be an apparent rather than a true form of assortative mating. Also, because large females would not be expected to prefer mating with small males, this pattern of larger mating females in 2006 may indicate that males are the choosy sex in *C. belfragei*. However, the fact that assortative mating was not detected in the field and the choice experiments found no difference in the preference of large versus small mates suggests that assortative mating may not be a characteristic of these two species.

Observational Experiment 2: Sexual Size Dimorphism

Both species exhibited female-biased SSD, but experiments did not implicate male choice of large females as a causal factor. The mean female size was significantly larger than the mean male size in both *C. belfragei* and *C. formosa*. Sexual size dimorphism theory predicts that in mating systems with high levels of male competition, sexual selection for larger male size will result in male-biased SSD. However, strong

male-male competition may still occur in species with female-biased size dimorphism, but in these species larger female size should only persist when selection for large female size is greater than selection for large male size (Arnqvist and Rowe 2005). In *C. belfragei* and *C. formosa* results from the male-male competition experiment showed that, although competition for the female was high, there was not an apparent large male mating advantage. This suggests that large female size outweighs selection for large male size in these species. An alternative, untested hypothesis, is that smaller males are favored due to reduced energy expenditures by mounted females. Males were observed in the mating chamber mounted on the female for up to 268 min in *C. belfragei* and up to 86 minutes in *C. formosa*. During these matings, females often moved around the mating area, obviously heavily laden with the mounted male. Also, in the field females were observed foraging with guarding males attached. If smaller males can maintain the same mate guarding benefits while reducing the costs to a particular female, then the evolution of smaller males should be favored (Arnqvist and Rowe 2005). Even if smaller males reduce female energy expenditures due to mating only by a fraction, this along with the lack of a large male advantage in male-male contests could explain the observed female-biased SSD.

It is important to note that sexual selection may be responsible for assortative mating and SSD in any given species, however, the causal factors involved in maintaining SSD may be difficult to identify. Assortative mating may fluctuate depending on current selective pressures, but SSD is not likely to fluctuate much over the short term (Andersson 1994). The relative merits of a given body size depend not only on present reproductive and other functions, but also on life history aspects such as time

to maturation and survival in relation to body size throughout ontogeny (Kozlowski 1989). Body size is related to many aspects of an organism's life, and determining the causes of such a general character may be difficult (Juliano 1992)

Observational Experiment 3: Mate Availability

Since date and site of collection had no effect on the mean size of males and females collected, the mate availability hypothesis can be excluded as an explanation of assortative mating and SSD. Eliminating mate availability as a factor increases the plausibility of sexual selection hypotheses of assortative mating and SSD.

Observational Experiment 4: Mating Behavior Sequence

The observed mating behavior sequence of both species occurred in three stages, similar to that described by Palmer (1976) for another tiger beetle species, *Pseudoxychila tarsus*. The main difference observed in *C. belfragei* and *C. formosa* was in phase 2. In phase 2 of mating instead of a complete withdrawal, *C. belfragei* and *C. formosa* withdrew the aedagus only partially, sometimes performing shallow pumps of the aedagus. These shallow pumps may serve to clear the lumen of the spermathecal duct before sperm transfer in phase 3. This lack of complete withdrawal of the aedagus in phase 2 was also documented in a study of two other tiger beetles in the genus *Cicindela* by Fielding and Knisley (1995). The specific male and female mating behaviors should relate to functional aspects of mating and can have a significant influence on sperm precedence and sexual selection strategies in tiger beetles. Previous work on tiger beetle mating structures revealed that the flagellum, a long thin sclerite in the internal sac of the aedagus of the male, is not connected to the ejaculatory duct and serves to open the

lumen of the spermatheca duct for sperm movement from the female bursa copulatrix (Freitag et al. 1980). The pumping of the aedagus in phase 2 observed in the genus *Cicindela* but not in *Pseudoxychila* may reflect a continuation of preparation of the lumen for sperm transfer, possibly by removing a previous male's ejaculate. This could indicate that sperm competition is more intense in the genus *Cicindela*, and males attempt to remove previous male ejaculates by shallow pumping of the aedagus in phase 2 of mating. These differences between phase 2 of mating may also be due to differences in female bursa copulatrix or spermatheca structure that result in a need for shallow pumping to remove previous ejaculates. In a study of the genitalia of four tiger beetles in the genus *Cicindela*, Freitag (1966) found evidence that suggested males transfer spermatophore constituents in a liquid form into the female bursa copulatrix from which a distinct spermatophore forms. The initial deep insertion of the aedagus and flagellum in phase 1 of mating may serve to puncture the spermatophore of a previous male, and the pumping observed in phase 2 of the genus *Cicindela* may be a means of facilitating the removal of previous ejaculates before sperm transfer. Also, the deep thrusts observed in *C. formosa* in phase 3 was not observed in *P. tarsus* or *C. belfragei*. This difference may reflect structural differences in male and female genitalia. The deep thrusts displayed by *C. formosa* may be necessary for sperm transfer to the bursa copulatrix, or may be part of male attempts to move sperm into the lumen of the spermatheca. Further, studies are required to test these hypotheses.

Why Should Male Choice and Male-Male Competition Not Coincide?

Crespi (1989) cited several studies that supported the idea that male choice and male-male competition are antagonistic processes that are not expected to coincide. First,

Manning (1980) found an inverse relationship between the strength of male-male competition and the efficacy of male choice in a study of *A. aquaticus* isopods. Second, in field populations of the cerambycid beetle *T. tetraophthalmus* with male-biased sex ratios, females were paired regardless of size and paired males were larger than unpaired males due to a large male advantage in male-male contests for females (Lawrence 1986). Conversely, in this same study it was discovered that when the sex ratio was female-biased, males were paired regardless of size but paired females were larger than unpaired females due to male choice of large females. Third, Borgia (1981) found no evidence of male choice in the dung fly *Scatophaga stercoraria*, which had intense male-male competition and did not mate assortatively in his study. Thus, when male-male competition is sufficiently high and the sex ratio is male-biased, selection may favor males that will pair with any female over males that pair with larger females.

Conclusions

Although size assortative mating did not occur in the field in either species, female *C. belfragei* in the field were larger in 2006 than non-mating females collected in the field, indicating that larger females may be preferred. Female-biased sexual size dimorphism was detected in both species, suggesting that selection for large females is greater than selection for large males.

Future Studies

Further study of male choice hypotheses for assortative mating and SSD require determining how male ability to choose large females varies with the intensity of male-male competition. Of course, the operational sex ratio, density, and mating system

characteristics must also be evaluated, as these factors influence the level of male-male competition at any particular time in any given population

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