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Plasticity in Female Mate Choosiness: A Result of Variation in Perceived Predation Risk and the Interaction of Female Age and Male Density

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PLASTICITY IN FEMALE MATE CHOOSINESS: A RESULT OF VARIATION IN PERCEIVED PREDATION RISK AND THE INTERACTION OF FEMALE AGE AND MALE DENSITY

by

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PLASTICITY IN FEMALE MATE CHOOSINESS: A RESULT OF VARIATION IN PERCEIVED PREDATION RISK AND THE INTERACTION OF FEMALE AGE AND MALE DENSITY

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In many species, female mate choices can be a strong source of sexual selection. Females often prefer a certain male phenotype, and this can be due to benefits females gain from mating with preferred males. However, such benefits can sometimes be outweighed by the cost of searching for a preferred male. These costs and benefits often change concomitantly with changes in environmental (e.g., predator abundance and conspecific density) and internal factors (e.g., female age). Thus, female mate choosiness (the degree to which preferences for certain males are expressed) should often be plastic. Plasticity in female mate choosiness may be complicated because environmental and internal factors may often interact naturally with one another. Therefore, it is important to understand how plasticity in mate choosiness is affected by such interactions. A small, yet growing, number of studies have investigated interactions on female mate choosiness, and have found interesting yet sometimes contradictory results. We conducted two experiments on the variable field cricket, *Gryllus lineaticeps.* One tested the effect of the interaction between female age (young, intermediate, and old) and male calling density (high and low), and the other tested the effect of the interaction of female age and perceived predation risk (predation chemical cues present or not) from sympatric, cursorial, wolf spider predators *Hogna* sp. Our results indicate a significant effect of the interaction between female age and male density, as well as a significant effect of perceived predation risk, on the plasticity of female mate choosiness. Young females exhibited the most plasticity in response to variation in male densities: young females were the most choosy in high male density environments and least choosy in low male density environments. Females, regardless of age, responded to variation in perceived predation risk the same: females were choosy when predation cues were absent, but decreased their choosiness when predation cues were present. Overall, these results suggest females who have the highest reproductive potential are the most responsive to changes in environmental and internal factors, but also that these factors can either interact with or overshadow each other to influence female mate choosiness.

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ABSTRACT

Female mate choices are commonly acknowledged to influence the strength and direction of evolution by sexual selection. Such choices are often plastic, adjusting in response to changes in environmental (e.g., predator abundance or conspecific density) and internal (e.g., female age) conditions. Though these factors can interact naturally, the effects of such interactions are only beginning to be studied. We used female variable field crickets, *Gryllus lineaticeps*, to examine how female age and perceived male density interact to affect the plasticity of a specific aspect of female mate choice: female choosiness. Female choosiness is a measure of female responses to male traits as they deviate from the female's preferred male trait; the more choosy a female is, the less responsive she will be to males as they deviate from the preferred value. We exposed females to either a high or low male calling density environment, then tested them at one of three different ages. We measured female mate choosiness by measuring the female's responsiveness to a low quality male song: the more choosy the female, the less time spent around the low quality male song. We found that female age and perceived male density interacted to significantly affect female mate choice plasticity. Young females were the most plastic, exhibiting the highest level of choosiness in high male density environments and the lowest in low male density environments. Intermediate and old females exhibited less plasticity and intermediate choosiness compared to young females.

Thus, sexual selection may be strongest at high male densities when most of the females are young.

INTRODUCTION

Phenotypic plasticity is an individual's ability to express different phenotypes in response to variation in internal physiology or the external environment (Stearns 1992; Widemo & Saether 1999; Anton et al. 2007; Bretman et al. 2011). A wide range of taxa show plasticity in morphology, growth, life history, and behavior (Miner et al. 2005; Anton et al. 2007), and such plasticity has the ability to influence the rate and direction of evolution (Price 2006; Thilbert-Plante & Hendry 2011). Behavioral plasticity is particularly interesting because behavior is often more reversible and labile than other traits (West-Eberhard 2003). And because behavior is an important source of selection, variation in behavior in response to changes in external or internal conditions can cause rapid changes in the nature of selection (West-Eberhard 2003; Duckworth 2008). For example, plasticity in female mating behavior, including female mate choosiness, can affect the strength and direction of sexual selection as it acts on male signals (Cotton et al. 2006; Rodríguez et al. 2013a, 2013b). External or internal conditions that cause females to mate indiscriminately may decrease the strength of sexual selection, thus slowing the spread of preferred male traits (Poulin & Vickery 1996) or even favoring less elaborate traits (Tinghitella & Zuk 2009). Alternatively, conditions that cause females to mate discriminately may increase the strength of sexual selection, thus increasing the preponderance of a preferred trait as quickly as a single generation (Kudo & Karino 2011).

Female choosiness is a measure of how strongly females respond to male trait values that deviate from the most preferred value; females that are more choosy respond strongly only to a narrow range of trait values, while females that are less choosy respond strongly to a broader range of trait values (Jennions & Petrie 1997; Gray & Cade 1999; Brooks & Endler 2001; Figure 1.1). Because choosiness can be costly (Andersson 1994; Brown 1999; Cotton et al. 2006), and because the costs can vary depending on external and internal conditions (Jennions & Petrie 1997), plasticity in mate choosiness may often be favored (Snell-Rood 2013). For example, choosier females may pay greater search costs in order to mate with males with the most preferred traits, while less choosy females may pay lesser search costs because they will mate with males with a broader range of traits. As a result, females may adjust their choosiness based on their ability to afford perceived search costs. Many studies have manipulated the costs of choosiness and have found that females adjust choosiness in response to changes in a variety of factors such as parasitism (Beckers & Wagner 2013), age (Richard et al. 2005; Ronald et al. 2012), body condition and diet (Hebets et al. 2008; Vitousek 2009; Moskalik & Uetz 2011), predation (Karino et al. 2000; Willis et al. 2012), male density and trait variability (Lehmann 2007; Fowler-Finn & Rodríguez 2012a), and previous experiences with certain male phenotypes (Wagner et al. 2001; Hebets 2003; Fowler-Finn & Rodríguez 2012b).

In the current study, we focused on the effects of female age and perceived male density on female mate choosiness. Females exhibit plasticity in choosiness in relation to age, typically (but not always) with younger females being choosier than older females (Gray 1999; Pervez et al. 2004; Richard et al. 2005). Life history theory predicts that a female's ability to invest in reproduction decreases as she ages: older females may not be able to afford the time or energy costs of searching for males with highly preferred traits, thus females should become less choosy as they age (Mautz & Sakaluk 2008; Cotter et al. 2011). Females also exhibit plasticity in choosiness in relation to male density, with increasing densities resulting in increased choosiness (Crowley et al. 1991; Lehmann 2007; Willis et al. 2011). When potential mates are abundant, females can afford to be choosier as the costs of rejecting males with less preferred traits are small. However, when potential mates are scarce, search costs may preclude high levels of choosiness (Shelly & Bailey 1992; Berglund 1995).

Most studies of plasticity have been conducted by manipulating one factor at a time (Nylin & Gotthard 1998; Bretman et al. 2011), and plasticity in female choosiness is no exception (see above, Wilgers & Hebets 2012; Tinghitella et al. 2013). However, females make mate choices in highly variable environments in which multiple factors can affect the costs of being choosy. These factors can interact, complicating the choices females must make (Cotton et al. 2006; Ronald et al. 2012). In order to understand how interactions between different factors can affect female choosiness, we simultaneously manipulated female age and perceived male density. A small number of studies have found that female choosiness can be influenced by such interactions. For example, females placed on high-quantity diets (Wilgers & Hebets 2012), or in male-biased social environments (Tinghitella et al. 2013), exhibited the same levels of choosiness, regardless of age. However, females on low-quantity diets, or in female-biased social environments, decreased choosiness as they aged. Other studies testing for interactions have found no evidence to support them; female choosiness responds to differences in mating history, but there was no effect of the interaction between mating history and either female age (Judge et al. 2010) or predation (Gabor & Page 2003).

We tested the effect of the interaction of female age and perceived male density on female choosiness in the variable field cricket, *Gryllus lineaticeps.* Field crickets are known to show plasticity in choosiness in response to age, with younger females being choosier and taking longer to make mating decisions than older females (Gray 1999; Mautz & Sakaluk 2008). Male *G. lineaticeps* calling densities fluctuate throughout the breeding season (Beckers & Wagner 2011), which can influence a female's response to a male call. Typically, the more exposure a female field cricket has to male calls, the choosier she becomes (Wagner et al. 2001; Bailey & Zuk 2008, 2009). Additionally, in *G. lineaticeps,* numerous studies have shown that females prefer males with higher chirp rates (Wagner 2011). Therefore, choosiness can be reflected by the amount of time a female spends in association with a very low chirp rate song; the less time females spend around a low chirp rate song, the more choosy the female (Wagner et al. 2001). We predicted there would be an interaction between female age and perceived male density: as perceived male density decreases, choosiness should decrease more for females of older ages (see Figure 1.2). In other words, as perceived male density decreases, the time spent near a low chirp rate song should increase more for females of older ages. We predicted this interaction pattern because we expected that older females, who already have a compromised ability to pay the search costs associated with time spent searching for a preferred male, would be more sensitive to changes in search costs associated with perceived male density. Young females, unlike old females, should be able to afford the time to search for a preferred male at lower densities.

METHODS

Study System

Adult female *Gryllus lineaticeps* were collected from the University of California Sedgwick Reserve in Santa Ynez, California in July 2012. They were shipped to the University of Nebraska – Lincoln and housed individually in plastic family containers (17.8 x 30.5 x 24.1 cm). The bottom of each container was lined with a paper towel, and included an egg carton for shelter, vermiculite for an egg-laying substrate, Purina® Complete Cat Chow® and ad libitum water (Wagner et al. 2001). Most wild caught females were gravid and laid eggs within 2-3 weeks of being in the laboratory. The hatched juveniles were kept and reared in the family containers until their 4th or 5th instar. At that time, juvenile females were removed from their family containers and placed individually in smaller plastic containers (10.8 x 17.8 x 14.0 cm) that held all of the above materials except vermiculite. The juvenile females in these smaller containers were transferred to an acoustically isolated room to control for the female's social experiences. We checked the smaller containers daily to determine when each female molted into the adult stage. On the day of the final molt, we assigned adult females to a treatment group (see below) and moved them to a separate isolation chamber. Each female was assigned only one treatment group, and each female was tested only once.

All rearing, treatment, and testing conditions were held constant, with a 14:10 hour reversed light:dark cycle, temperature at 20-22 C°, and humidity between 30-60%. All females were the first generation offspring of wild-caught females. Testing occurred from April 5, 2013 until June 7, 2013.

Experimental Treatments

We manipulated age by testing the female at either 13 days (Young), 19 days (Intermediate), or 25 days (Old) following the final molt. Female field crickets are usually not responsive to mating until 7-10 days after their adult molt and rarely live more than four weeks under natural conditions (Zuk 1988; Murray & Cade 1995; Judge et al. 2010). The ages of the females tested thus spanned the natural range of female ages. We manipulated perceived male density by broadcasting either three (Low Density) or nine (High Density) male songs to females six days prior to testing. Thus, there were a total of six treatment groups: Low Density x Young Age, Low Density x Intermediate Age, Low Density x Old Age, High Density x Young Age, High Density x Intermediate Age, and High Density x Old Age.

The male songs used for the density manipulation consisted of the nine possible combinations of three chirp rates (1.8, 3.0, and 4.2 chirps/second) and three chirp durations (90, 120, and 150 ms). These parameters span the natural range of male *Gryllus lineaticeps* songs (Wagner & Reiser 2000; Wagner & Basolo 2007). The same nine songs were broadcast in both the High Density and Low Density treatments to control for song variability between density treatments. All nine songs were broadcast each day during the High Density treatment, while three sets of three songs were rotated each day for the Low Density treatment. The songs were broadcast simultaneously for eight hours during the female's dark cycle for each of the six days prior to testing, starting one hour after the dark cycle began and stopping one hour before the dark cycle ended. We broadcast each song from a separate speaker, and each speaker was placed 75 cm apart and arranged in an arc two meters from the center of the shelving unit where the females were placed. Songs were broadcast at 70 dB SPL (re: 20 µPA) measured 30 cm from the edge of the speaker using a Cel-254 dB sound level meter. Small pieces of cardboard were placed between female containers to visually isolate the females, and the shelf positions of the females were rotated each day to give exposure to different song micro-environments.

The density treatments were conducted in a single room, therefore the High Density and Low Density treatments could not be conducted simultaneously. To ensure that the time period did not confound the density manipulation, but to also accommodate for six-day treatments and variable female maturation times, we alternated High Density and Low Treatments in two-week blocks.

Measuring Female Choosiness

Female responses were tested during the dark cycle at 20-22 ºC. To measure female choosiness, we conducted one speaker tests in a square, semianechoic chamber measuring 2.2 x 2.2 x 2.7 m (length x width x height) (Beckers & Wagner 2011). We controlled for the speaker location by randomly assigning the speaker to one corner of the chamber before each test began, and randomly assigning within treatments to ensure balance between speaker locations and treatments. We broadcast a slow chirp rate/short duration male song (1.8 chirps/second, 90 ms) from the speaker, which female *Gryllus lineaticeps* perceive as low quality (Wagner 1996; Wagner & Harper 2003). The song was broadcast at 70 dB SPL (re: 20µ Pa) at 30 cm from the speaker, measured using a Cel-254 dB sound level meter. The song was broadcast from a CD using a Sony CD Walkman D-EJ011.

The male song was broadcast for 20 minutes. The broadcast began when we placed the female cricket in the center of the chamber under a cup for a five-minute acclimation period. After the acclimation period, the female was released for a 15-minute test. We measured the amount of the time the female spent within 26 cm of the speaker (which will be referred to as the Choice Zone). Association time is correlated with actual female mate choice (White & Galef 1999; Morris et al. 2010; Beckers & Wagner 2011) and has been used as a measurement of choosiness (Amcoff et al. 2013). Because the test song was a highly non-preferred song type, females were considered less choosy the more strongly they responded. After each trial, the arena was wiped down with a 10% isopropynol solution to remove any possible chemical cues left behind by the female cricket (see Gray 1999; Champagnon & Cueva del Castillo 2008).

Seven days after testing, each female was checked to ensure that she was alive. If she died, her data were discarded because her behavior during the test could have been a result of senescence (n = 1). Females were also discarded if they jumped on the walls of the chamber, fluttered their wings (i.e., attempted to fly), or never moved after the acclimation period (n = 26). If females exhibited these behaviors, it was assumed the female would not be receptive to any male song, therefore choosiness could not be measured. We also did not test females that were physically damaged (e.g., females with missing legs or broken antennae) (n = 24).

Binomial regression was used to determine whether females were more likely to be discarded in certain age or density treatments due to the aforementioned behavioral or physical reasons. There were no differences in total discarded females between any of the treatments ($z \leq 1.84$, $P \geq 0.066$). However, there was a significantly smaller propensity for females to be discarded due to behavioral issues (see above) the older they were ($z = 2.17$, $P = 0.030$), regardless of density treatment. Old females attempted to fly (n = 1) or exhibited other behaviors (n= 2) less often than Intermediate (attempt to fly: n = 6, other: n = 1) or Young (attempt to fly: n = 10, other: n = 2) females.

A total of 172 females were tested successfully in the six treatments: Low Density x Young Age (n=24), Low Density x Intermediate Age (n=23), Low Density x Old Age (n=35), High Density x Young Age (n=29), High Density x Intermediate Age (n=28), and High Density x Old Age (n=33). On average, 1.25 females per family were successfully tested per treatment.

Statistics

Analyses were conducted in Stata 13.0 for Mac. To evaluate effects of female age and perceived male density, we compared the amount of time females spent in the Choice

Zone using a zero-inflated negative binomial regression (ZINB). We used ZINB because the response variable data had overdispersion and an excess of zeros that derive from one of two processes (Ridout et al. 2001): a female could have spent zero time in the Choice Zone because she never would have chosen the low quality song, or because the 15-minute trial did not allow enough time for a female to choose the low quality song. The independent variables in the model were age, density, and age x density. Because we tested females at three different ages, we also included a quadratic age term to test for a non-linear effect of age and a non-linear interaction between age and density. Inflated terms were age and density. Variables that were not experimentally controlled (i.e., the time the test began, the temperature at which the test was run, and female mass) were also included in the initial model. We included family in the initial model as a fixed effect because up to five females in each treatment were from a full sibling family. Pairwise comparisons were made between density treatments within each age (for a total of 3 comparisons) and the alpha level for significance was Bonferroni corrected to $P < .017$.

RESULTS

There was a non-significant effect of time ($X^2_1 = 2.01$, $P = 0.157$), temperature ($X^2_1 = 0.31$, $P = 0.577$), mass ($X^2_1 = 1.61$, $P = 0.205$), and family ($X^2_1 = 0.68$, $P = 0.408$) on female choosiness. These variables were thus dropped from the model.

The time females spent in the Choice Zone was significantly influenced by the quadratic age term ($X^2_1 = 5.91$, $P = 0.015$, Table 1.1, Figure 1.3) and the interaction between the quadratic age term and density ($X^2_1 = 6.13$, $P = 0.013$, Table 1.1, Figure 1.3).

Young females exhibited the greatest plasticity in choosiness; they spent significantly more time in the Choice Zone (i.e., were least choosy) in the Low Density environment compared to in the High Density environment ($z \leq 3.16$, $P \geq 0.002$). In contrast, Intermediate and Old females showed little change in choosiness between density environments ($z \leq 1.85$, $P \geq 0.064$).

DISCUSSION

Our goal was to investigate how perceived male density, female age, and their interaction affect female mate choosiness. Our results indicate nearly the opposite of our prediction: Young females were the most plastic in their mate choosiness in response to changes in perceived male density. They were the least choosy in low male density environments, but the most choosy in high male density environments.

Studies testing how interactions between factors affect female mate choosiness have produced highly variable results. Significant interaction effects have been found in fish (female age and operational sex ratio, Tinghitella et al. 2013), spiders (female age and female diet, Wilgers & Hebets 2012), amphibians (call type, call amplitude, perceived call distance, and light levels, Bonachea & Ryan 2011), and insects (time in mating season and predation risk, Lafaille et al. 2010; female age and perceived male density, this study). Some studies have tested for interactions, but found only a main effect of one factor (female age but not nutritional condition or body size, Gray 1999; mating status but not female age, Judge et al. 2010; female age but not mating status, Mautz & Sakaluk 2008) or no effects at all (female isoline and ambient temperature, Ingleby et al. 2013). To our knowledge, this is only one of three studies to examine the effect of the interaction between female age and male availability on female mating behavior. Tinghitella et al (2013) manipulated both operational sex ratio (the ratio of sexually receptive females to competing males, thus the environment was either female or male biased) and female age in stickleback fish. They found a significant effect of the interaction between these factors; older females were less choosy than young females, but only in female-biased environments. Old and young females in the male-biased environments did not differ in choosiness. Tinghitella (2014) also manipulated density and female age in a cricket, *Acheta domesticus,* and found that older females approached males more often than younger females. However, females in low and high density environments responded to males similarly, and there was no effect of the interaction between female age and density. Female mating decisions, such as latency to mount a male, were not affected by density, age, or their interaction. These contrast with our result, in which younger females were the least choosy in the low male density environment, but also the most choosy in the high density environment.

One explanation for the differences between the results of this study and Tinghitella et al. (2013) involves differences in the life histories of the study organisms. It can be expected that females with the most reproductive potential should exhibit a greater degree of behavioral plasticity in response to changes in conditions (Lafaille et al. 2010; Fischer et al. 2014). Because females with more reproductive potential have more fitness to lose if they do not gain matings, they should be more willing to accept lower quality males if perceived male availability is low. In many insects, including field crickets, older females typically produce fewer eggs than young females, thus older females typically have less reproductive potential (Foster & Howard 1999; Kindlmann et al. 2001; Omkar & Pervez 2002; Evenden et al. 2006). In contrast, many fish exhibit the opposite pattern. Older females, which are typically larger than young females due to continued growth after maturation, produce more eggs than young females and, therefore, typically have more reproductive potential (Nieland & Wilson 1993; Bobko & Berkeley 2004). Thus, the results of the two studies may be consistent: the ages that have the most reproductive potential to lose are often those that are least choosy when male availability is low. One reason why our results might contrast with Tinghitella (2014) is due to the differences in the responses measured. While we measured time investment as an indicator of choosiness, Tinghitella (2014) measured female approaches to males as an indicator of interest in mating, as well as latency to mount a male. It is possible that the time invested in searching for a male may not directly correlate to the probability of approaching a male or the latency to mount a male once found. For example, females that approach males to better evaluate the males' quality may vary in the time spent searching for, approaching, and evaluating them.

Interestingly, our results are also consistent with studies looking at how reproductive potential interacts with predation (Magnhagen 1990; Candolin 1998; Lafaille et al. 2010). In a high predation risk environment, individuals with less reproductive potential to lose took more risks and were less plastic. In contrast, individuals with the most reproductive potential to lose decreased choosiness in high predation environments and exhibited the most plasticity. Because mate availability and predation have both been shown to affect female mate choosiness in similar ways, future studies investigating interactions between these two factors will be helpful in disentangling how they may jointly affect female mate choosiness, or whether one may be a stronger selective factor than the other.

While female choosiness was significantly affected by the interaction between age and density, there were no individual effects of age or density on female choosiness. This is surprising as age is thought to be an important factor in determining choosiness (Pianka & Parker 1975; Bretman et al. 2011; Ronald et al. 2012; Fischer et al. 2014). It has been shown, however, that prior reproductive experiences in field crickets can overshadow possible effects of age (Judge et al. 2010). While reproductive experience and age should often covary because older females usually have had more matings, we held reproductive experience constant for our study (i.e., all the females tested were virgins). It is also surprising that we found no effect of density, except in the interaction with age. Population densities can fluctuate throughout a season (Souroukis & Cade 1993; Ritz & Köholer 2007), and density has been shown to have an important effect on mating behavior and the strength of sexual selection (Lehmann 2007; Willis et al. 2011; Kasumovic & Brooks 2011). However, our results, and those of Tinghitella et al. (2013), suggest that females of differing ages respond very differently to variation in male density and availability. Thus, whether male density or availability are found to affect female choosiness may strongly depend on the age of the females tested.

Because younger female *G. lineaticeps* show greater plasticity in choosiness in response to variation in male density, the effect of density on the strength of sexual selection should depend on the age structure of the population. While female field crickets in natural populations can be older than four weeks of adult age, most are between seven and 15 days of adult age (Zuk 1987; Simmons 1995; Murray & Cade 1995). Consequently, changes in density should strongly affect the strength of sexual selection (Kokko & Monaghan 2001; Ryder et al. 2012; Bertram et al. 2013); as male density decreases so does female choosiness, weakening sexual selection. The implications for field populations could then be selection for phenotypic plasticity in both female and male mating behavior. Early and late in the breeding season, when adult densities of *G. lineaticeps* are low, our results suggest that young females are less choosy and thus that sexual selection should be weak. This should favor reduced male investment in expensive song characteristics such as high chirp rate. In contrast, in the middle of the breeding season, when adult densities of *G. lineaticeps* are high, our results suggest that young females are more choosy and thus that sexual selection should be strong. This should favor increased male investment in expensive song characteristics. Indeed, there is some evidence that male field crickets adjust their singing effort dependent upon mating activity, singing more often and more attractively when mating activity is highest (Bertram et al. 2013).

Although our result supports the common hypothesis that sexual selection should be stronger when male density is higher (Kokko & Rankin 2006), there seems to be no general theory of how density should affect the strength of sexual selection (Kokko & Rankin 2006; Sharp & Agrawal 2008). Studies that have examined the effect of density on the strength of sexual selection have produced variable results; nearly half have found stronger sexual selection at high densities, while nearly half have found stronger sexual selection selection at low densities (Sharp & Agrawal 2008). Our results suggest that the effect of density on the strength of sexual selection may depend, at least in part, on how females of the predominant age class adjust their choosiness to changes in male densities.

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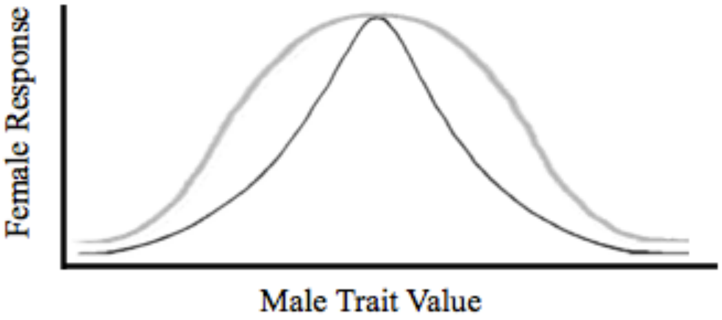


Figure 1.1. Theoretical representations of female choosiness. Females that are more choosy respond strongly to only a narrow range of male trait values (black line). Females that are less choosy respond strongly to a wider range of male trait values (grey line).

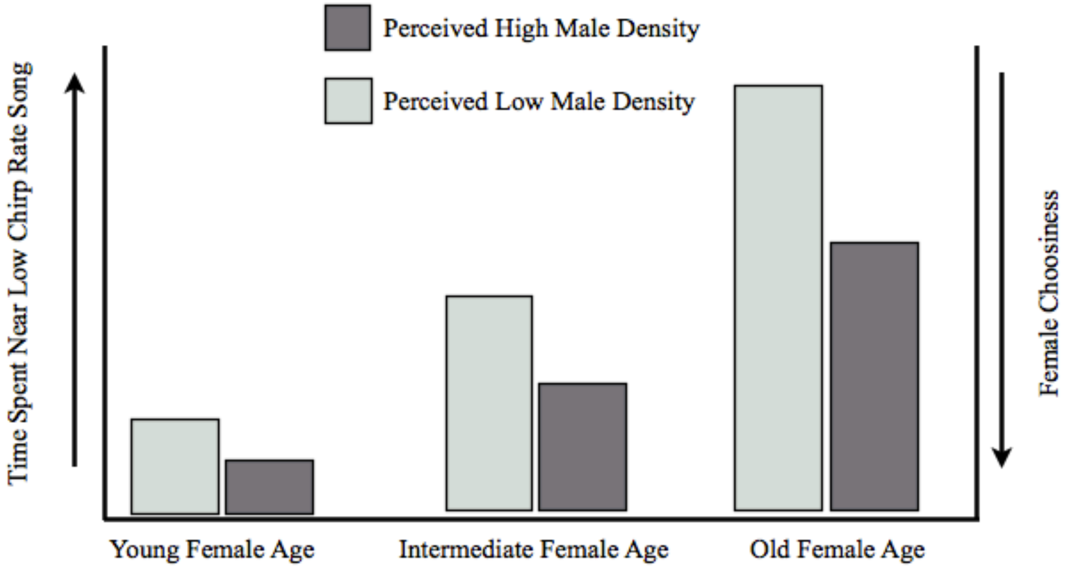
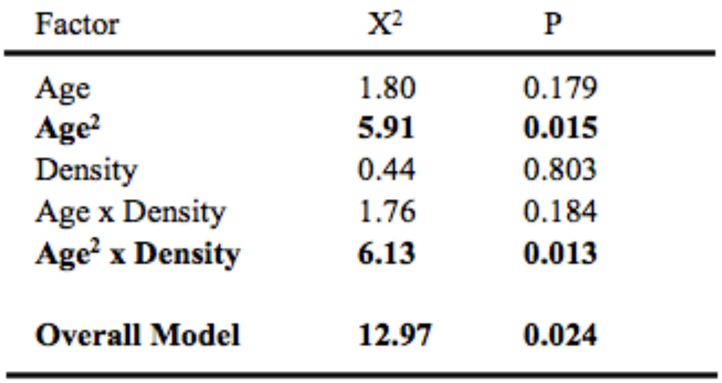


Figure 1.2. Predictions for female choosiness responses to the interaction of female age and perceived male density. We predicted that, as perceived male density decreases, choosiness decreases more for females of older ages. This is exhibited by females in low density spending more time near a low chirp rate song as they age.



Factor	X^2	P
Age	1.80	0.179
Age^2	**5.91**	**0.015**
Density	0.44	0.803
Age x Density	1.76	0.184
Age^2 x Density	**6.13**	**0.013**
Overall Model	**12.97**	**0.024**

Table 1.1. Results from the zero-inflated negative binomial model testing effects on female mate choosiness. Significant results are indicated in bold.

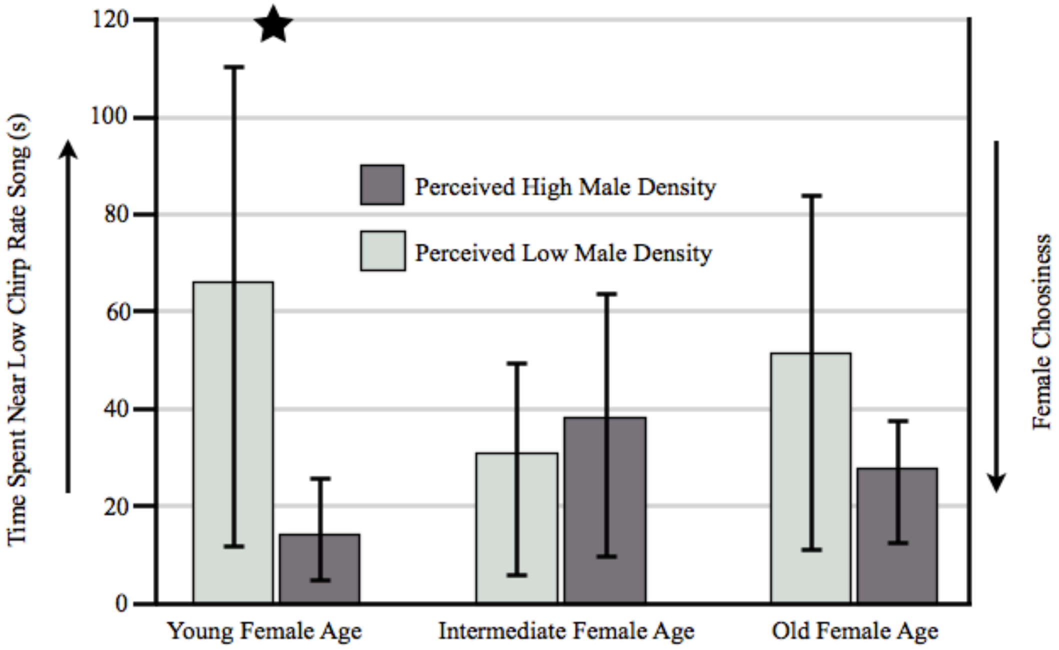


Figure 1.3. Mean time females spent near a low chirp rate song. The more time females spent near the song, the less choosy the females. Stars indicate significant differences between High and Low Male Density within the age group. Bars indicate 95% confidence intervals.

Chapter 2. Along Came a Spider Who Sat Down Beside Her: A Study of the Effect of Predation on the Plasticity of Female Mate Choice

ABSTRACT

Organisms often exhibit behavioral plasticity in response to changes in environmental (e.g., predation risk or mate density) and internal (e.g., age) conditions. Particularly, female mate choosiness (the strength of female's attraction to male traits as they deviate from preferred trait values) has repeatedly been shown to be plastic due to the costs associated with searching for preferred males concomitantly changing with environmental and internal conditions. Because environmental and internal factors can interact naturally, it is important to understand how female mate choosiness responds to these interactions. We studied the interaction between perceived predation risk and female age on the variable field cricket, *Gryllus lineaticeps*. Females were exposed to either no predation cues or predation cues from a sympatric, cursorial, wolf spider predator, *Hogna* sp. We then tested the females at one of three adult ages and measured their choosiness by recording their responsiveness to a low quality male song. We found female choosiness plasticity was affected by neither age nor the interaction between age and perceived predation risk. Perceived predation risk was the only factor to significantly affect the plasticity of female mate choosiness: females were less choosy when they perceived predation risk and were more choosy when they did not. Predation may be such a strong factor of selection that, regardless of differences in other factors, most all individuals respond nearly equally.

INTRODUCTION

Sexual selection can play a major role in the evolution of life history, morphological, and behavioral traits for many organisms (Andersson 1994). However, it has been posited that the strength and direction of sexual selection, as it acts through female mate choice, can often change (Cotton et al. 2006; Duckworth 2008). Females typically prefer a certain male phenotype (Andersson 1994; Andersson & Simmons 2006), but costs are often associated with searching for preferred males (Cotton et al. 2006). When females can afford the costs of searching for preferred males, they are said to be choosy because they are likely to only accept males with preferred traits. Choosy females could therefore increase the strength of sexual selection (Kudo & Karino 2011). But when costs of searching for a preferred male increase to a level which females cannot afford, females will often become less choosy and accept less preferred males. Less choosy females could therefore decrease the strength (Poulin & Vickery 1996) or reverse the direction (Tinghitella & Zuk 2009) of sexual selection. Female mate choosiness can thus be defined as the measure of female acceptance of male traits that deviate from the preferred phenotype (Jennions & Petrie 1997; Gray & Cade 1999; Brooks & Endler 2001; Figure 2.1). Female mate choosiness is often not static because search costs that influence choosiness can depend upon changes in internal and environmental conditions (Jennions & Petrie 1997). Thus, plasticity (the expression of different phenotypes in response to changes in conditions (Stearns 1992; Widemo & Saether 1999; Anton et al. 2007; Bretman et al. 2011)) in female mate choosiness should often be favored (Snell-Rood 2013). However, we are only beginning to understand how interactions between internal and environmental conditions influence search costs and thus female mate choosiness.

Plasticity in female choosiness is commonly expressed in response to variation in a variety of internal and environmental factors such as parasitism (Beckers & Wagner 2013), previous experiences with certain male phenotypes (Collins 1995; Hebets 2003), body condition and diet (Hebets et al. 2008; Vitousek 2009; Moskalik & Uetz 2011), and time in the season (Backwell & Passmore 1996; Milner et al. 2010). However, female mate choices are naturally made in highly variable environments, thus they are potentially affected by multiple, interacting factors (Cotton et al. 2006; Ronald et al. 2012). For instance, females have been shown to exhibit plasticity in choosiness dependent upon age, but only when on low-quantity diets (Wilgers & Hebets 2012) or in female-biased social environments (Tinghitella et al. 2013). Female mate choosiness has also been shown to be influenced by changes in male density, but only when females are young (Chapter 1), or changes in male density or female age, but not their interaction (Tinghitella 2014). Therefore, our goal in this study was to add to this small, yet growing, body of knowledge and assess how interactions can affect the plasticity of female mate choosiness. Specifically, we were interested in the interaction between perceived predation risk and female age.

Predation can have pervasive effects on prey traits (Endler 1986), including female mate choosiness. Females are commonly known to decrease their mate choosiness in response to increasing levels of perceived predation risk (Breden & Stoner 1987; Forsgren 1992; Karino et al. 2000; Willis et al. 2012). This is often attributed to the increased costs, normally in the form of the immediate risk of death (Kavaliers & Choleris 2001), associated with increased predation risk while searching for mates (Hedrick & Dill 1993; Gabor & Page 2003; Dunn et al. 2008; Bonachea & Ryan 2011). As such, a prey's ability to accurately assess cues of predation risk, and thus potential costs of mate searching, should be selected for (Lima & Dill 1990; Kavaliers & Choleris 2001). Female mate choosiness has also been shown to decrease with age (Gray 1999; Stout et al. 2010) due to a decreased ability to invest in searching for preferred males (Cotter et al. 2011). However, when the risk of death increases, it can be expected that older females may exhibit less plasticity in female mate choosiness than younger females. Older females often have less future reproduction opportunities, thus they often invest more in current reproduction efforts (e.g., they are more choosy than younger females) while younger females often invest more in surviving to increase future reproductive opportunities (e.g., they are less choosy than older females) (Magnhagen 1991; Candolin 1998; Lafaille et al. 2010).

To test the effects of the interaction between perceived predation risk and female age on the plasticity of female mate choosiness, we used the variable field cricket, *Gryllus lineaticeps,* and a sympatric, cursorial wolf spider predator, *Hogna* sp. Field crickets are commonly known to adjust various aspects of their physiology (Adamo & Baker 2011) and behavior (Kortet & Hedrick 2004; Hedrick & Kortet 2006) to changes in perceived predation risk, including their mate choice behavior (Hedrick & Dill 1993). Where *G. lineaticeps* and *Hogna* sp occur in sympatry, *Hogna* sp can be found at both relatively high (one spider per 3.3 m^2) and low (one spider per 27.5 m^2) densities (Atwell, Wagner, and Stafstrom; unpublished data). Therefore, *G. lineaticeps* females are naturally exposed to a predation gradient easily replicated in an experimental setting. Female field crickets have also been shown to exhibit plasticity in mate choosiness as they age; younger females are typically choosier and take more time in making mating decisions (Gray 1999; Mautz & Sakaluk 2008). Thus, *G. lineaticeps* and its predator, *Hogna* sp, are an excellent model system to study the interaction of perceived predation risk and female age. Additionally, *G. lineaticeps* is ideal for studying plasticity in female mate choosiness. Female *G. lineaticeps* are known to prefer higher male chirp rates (Wagner 2011), and choosiness can be measured by the acceptance of lower chirp rates; the more time a female spends around a low chirp rate call, the less choosy the female (Wagner et al. 2001, Chapter 1). We predicted that perceived predation risk and female age would have an interacting effect on female mate choosiness. Specifically, younger females should be more plastic in their mate choosiness than older females (Figure 2.2). Young females should decrease their choosiness when they perceive predation risk so that they increase their chances of survival and future reproduction. Older females should be less plastic because they have fewer future reproductive opportunities to lose in a predation environment.

METHODS

Study System

Adult *Gryllus lineaticeps* were collected in August 2012 from the University of California Sedgwick Reserve, Santa Ynez, California. After being shipped to the

University of Nebraska – Lincoln, each adult female was placed in a separate, large, plastic container (17.8 x 30.5 x 24.1 cm). Containers were lined at the bottom with a paper towel and included Purina® Complete Cat Chow® and water ad libitum, an egg carton for shelter, and vermiculite as an egg-laying substrate (Wagner et al. 2001). Most wild caught females were gravid and began laying eggs within two to three weeks. F1 offspring from each female were raised in the large container until they reached their 4th or 5th instar, at which time they were separated individually into small containers (10.8 x 17.8 x 14.0 cm). These small containers held the same materials as the large containers except vermiculite. Mating pairs of the F1 generation were managed to decrease the possibility of inbreeding (Beckers & Wagner 2011) and were placed into new, large containers to produce the F2 generation. When female F2 offspring reached their 4th or 5th instar, we separated them individually into small containers, placed them in an acoustically isolated juvenile chamber, and checked them every day for maturation. Upon maturation, females were randomly assigned a treatment (see below) and moved to an acoustically isolated adult female chamber until treatments began. Each female was assigned a single treatment and was thus only tested once.

The crickets were maintained and tested at a constant 14:10 hour reversed light:dark cycle at 20-22°C and a relative humidity of 20-80%. All females were tested between August 28, 2013 and November 23, 2013.

Adult female *Hogna* sp. were collected from the University of California Sedgwick Reserve in July 2013. These spiders consume adult *G. lineaticeps* readily in the field and in the lab (Atwell, Wagner, and Stafstrom, personal observation). The spiders were shipped back to the University of Nebraska – Lincoln where they were housed individually in plastic containers (9.0 x 11.5 x 9.0 cm) and fed one juvenile *G. lineaticeps* every three days and given water ad libitum. The spiders were kept in a climate-controlled chamber that was separate from the adult female crickets but held under the same conditions.

Experimental Treatments

We manipulated perceived predation risk by exposing female crickets either to Predation chemical cues orNo Predation chemical cues, and we then tested female choosiness at one of three ages. Thus, we created six treatments: Young Age x Predation, Young Age x No Predation, Intermediate Age x Predation, Intermediate Age x No Predation, Old Age x Predation, and Old Age x No Predation.

To test the effect of female age, females were tested when they were either young (10 days after their final molt), intermediate (20 days after their final molt), or old (30 days after their final molt). Our age categories were based on previous research showing adult female crickets are usually responsive to mating starting at about 7-10 days of adult age and normally are not older than about four weeks of adult age in the field (Zuk 1987; Murray & Cade 1995; Judge et al. 2010). We have also shown an effect of an interaction between female age and male density on female choosiness when using females near this age range (Chapter 1).

We manipulated perceived predation risk by placing a piece of filter paper, with or without predation chemical cues, inside a female cricket's container. Previous studies have shown that other field cricket species are capable of detecting and adjusting their behavior to wolf spider cues (including silk, excreta, and secretions) collected on filter paper (Kortet & Hedrick 2004; Storm & Lima 2008). To collect cues, half of a 90 mm circular filter paper (hereafter called Cue Paper) was placed for three days in a Predation cue container or a No Predation cue container (9.0 x 11.5 x 9.0 cm). Preliminary data showed no effect of predation on *G. lineaticeps* female mate choosiness when the filter paper was held in the containers for one day ($X^2_2 = 0.19$, $P = 0.908$), thus we decided to gather cues for three days. We collected cues on two Cue Papers on each of two adjacent sides of the container, for a total of four Cue Papers per container. The other two sides of the container held mesh. In order to ensure that cues were evenly distributed between the Cue Papers, the containers were rotated twice daily so that each side with Cue Paper was on the bottom for the same amount of time. We monitored the location of spiders twice a day for eight days and found 79.7% of the spiders on Cue Paper at any given time.

The Predation cue containers held a spider, as well as a juvenile *G. lineaticeps* for food. A new container and juvenile *G. lineaticeps* were provided every three days when the Cue Paper was collected and replaced. The Predation Cue Paper potentially collected predation cues not just from the spider, but also from the juvenile *G. lineaticeps* both before and after its consumption by the spider. To control for *G. lineaticeps* juvenile cues other than predation-related, the No Predation cue containers held a single, live juvenile *G. lineaticeps.* Once the Predation or No Predation cues were collected, we exposed female *G. lineaticeps* to the cues by placing the Cue Paper inside a female cricket's container for six days prior to testing. To simulate a natural pattern of intermittent encounters with spider cues, the Cue Paper was placed in a female's container for 30 minutes at a random time during their dark cycle.

We began this experiment with a total of 16 spiders to collect predation cues from: three sets of four spiders and three extra to replace any that died. Over the course of this three month experiment, 12 spiders died, most likely due to old age. The spiders were collected as adults in the field, and were collected two months before the experiment began. Thus, at the youngest, they were five months of adult age at the completion of the experiment. However, female cricket mate choosiness was not affected by spider aging, senescence, or replacement throughout the study ($t = 0.35$, $P = 0.726$).

To simulate natural conditions during the treatments, we played six male songs simultaneously that varied in chirp rate and chirp duration for eight hours during the female's dark cycle for each of the six days prior to testing: 1.8 chirps/second, 90 seconds; 1.8 chirps/second, 120 seconds; 3.0 chirps/second, 90 seconds; 3.0 chirps/second, 150 seconds; 4.2 chirps/second, 120 seconds; 4.2 chirps/second, 150 seconds. The male songs spanned the natural range of *G. lineaticeps* (Wagner & Reiser 2000; Wagner & Basolo 2007), and were, on average, the average chirp rate and duration (3.0 chirps/second, 120 seconds). Under natural conditions, adult female *G. lineaticeps* rarely experience silence before finding a male, and under experimental conditions, females are much less choosy after being held in silence (Beckers & Wagner 2011). We broadcast each song from a separate speaker, and each speaker was placed 75 cm apart and arranged in an arc two m from the center of the shelving unit where the females were placed. Songs were broadcast at 70 dB SPL (re: 20 µPA) measured 30 cm from the edge of the speaker using a Cel-254 dB sound level meter. Additionally, cardboard dividers visually isolated the females from each other and the females were rotated each day on the shelving unit.

Measuring Female Choosiness

We tested females during their dark cycle the day after their sixth day of treatment. We measured female choosiness by broadcasting a single male song from a speaker in a corner of a semianechoic chamber (2.2m width x 2.2m length x 2.7m height) (Wagner & Basolo 2007; Beckers & Wagner 2011). The song consisted of a slow chirp rate and short chirp duration (1.8 chirps/second, 90 ms), which is perceived as a low quality male song by female *G. lineaticeps* (Wagner 1996). The song was played from a Mac Mini using Amadeus Pro version 2.1.3. We broadcast the song at 70 dB SPL (re: 20 μPA) measured with a Cel-254 dB sound level meter 30 cm from the front of the speaker. The speaker placement was chosen at random before each test began, and the location was randomized within treatments to ensure balance between speaker locations and treatments.

A female was placed in the middle of the chamber under a cup and we broadcast the song for 20 minutes. The first five minutes were designated as the acclimation time, during which the female remained under the cup. The female was then released for a 15 minute trial, during which the female was free to move around the floor of the testing chamber. Because association time has been found to correlate with female choice (White & Galef 1999; Morris et al. 2010; Beckers & Wagner 2011), we measured choosiness as the amount of time a female spent within 26 cm of the broadcasting speaker (known as the Choice Zone) (Wagner et al. 2001; Wagner & Basolo 2007; Beckers & Wagner 2011, 2013). The less time a female spent in association with the low quality male song, the more choosy we considered the female (Chapter 1). At the completion of the trial, the floor of the arena and the speaker were wiped down with 10% isopropynol to control for any cues the female cricket may have left behind (see Gray 1999; Champagnon & Cueva del Castillo 2008).

The females were checked seven days after testing to ensure that they were still alive. If a female had died, her data were discarded since her response could have been a product of her senescence rather than the treatment (n = 1). Females were also discarded if they exhibited behaviors indicating they were not receptive to any male song (thus, choosiness could not be measured), including: jumping onto the walls of the chamber, attempting to fly, or never moving from the center of the chamber where the acclimation period took place (n = 21). We did not test physically damaged females (e.g., malformed or missing legs or wings, broken antennae, etc.) (n = 31).

A binomial regression was used to determine whether females of a given treatment had different propensities to be discarded due to behavior or physical damage. There were no differences between the six treatments for the overall propensity to be discarded ($z \leq 1.87$, $P \geq 0.062$). However, older females (regardless of predation treatment) were significantly less likely to be discarded due to behavioral problems ($z = 2.40$, $P = 0.016$). Old females attempted to fly (n = 2) or exhibited other behaviors (n= 1) less often than Intermediate (attempt to fly: n = 4, other: n = 2) or Young (attempt to fly: n = 6, other: n = 5) females.

A total of 158 females were successfully tested: Young x Predation (n = 24), Young x No Predation (n = 30), Intermediate x Predation (n = 24), Intermediate x No Predation (n = 26), Old x Predation (n = 27), and Old x No Predation (n = 27). On average, 1.05 females per family were successfully tested per treatment.

Statistics

All statistics were run on Stata 13.0 for Macintosh. We used a zero-inflated negative binomial (ZINB) regression to evaluate choosiness by comparing the amount of time in seconds that females spent in the Choice Zone. A ZINB was used because the data was overdispersed and had an excess of zeros that came from one of two processes (Ridout et al. 2001). The two processes that lead to the excess of zeros (when a female never went into the Choice Zone during the trial) were: a female was choosy enough that she never would have entered the Choice Zone, or a female was choosy but would have entered the Choice Zone if given longer. The initial model included Age, Predation, and their interaction as independent variables. Inflated terms were Age and Predation. We also included variables that were not experimentally controlled for (i.e., the time the test began, the temperature at which the test was run, and female mass).

We used a separate ZINB regression to analyze the effect of family on female responses, as one to four females in each treatment were from a full sibling family. Time spent in the Choice Zone was the dependent variable and family was the only independent variable as well as the inflated term. Family did not have a significant effect on time spent in the Choice Zone ($X^2_2 = 3.74$, $P = 0.155$).

RESULTS

Female choosiness was not significantly affected by time of day ($X^2_1 = 1.42$, $P = 0.233$), temperature ($X^2_1 = 0.05$, $P = 0.823$), or mass ($X^2_1 = 1.06$, $P = 0.303$). Thus, these variables were dropped from the model. The interaction between perceived predation risk and female age was also not significant ($X^2_3 = 1.73$, $P = 0.630$, Figure 2.3), and was also dropped from the model. Therefore, the final model included perceived predation risk and female age as independent variables.

Female choosiness was significantly affected by perceived predation risk ($X^2_2 = 6.04$, $P = 0.014$, Figure 2.4) but not by female age ($X^2_2 = 0.90$, $P = 0.344$). Females in the Predation treatment spent significantly more time in the Choice Zone around the low quality male song, and were thus less choosy, than females in the No Predation treatment, regardless of their age.

DISCUSSION

Contrary to our predictions, female mate choosiness was affected only by perceived predation risk; females spent more time around the low quality song, and were thus less choosy, in perceived predation risk environments. This indicates that perhaps predation is such a strong selective factor that most females, regardless of differences in age, adjust choosiness nearly the same.

Results from similar studies have commonly found a somewhat different pattern: predation risk often affects mating behavior, but unequally between individuals with different future reproductive potential. Responses to changes in predation risk are typically stronger for individuals with more future reproductive potential, such as younger individuals (Magnhagen 1990), smaller individuals (Candolin 1998), or individuals earlier in the mating season (Lafaille et al. 2010). Though these individuals can otherwise afford to invest the most in reproductive efforts (Backwell & Passmore 1996; Olvido & Wagner 2004), they also have the most reproductive potential to lose if they are consumed by a predator (Candolin 1998; Lafaille et al. 2010). Thus, these individuals typically decrease risk-taking behavior at the expense of current reproduction in order to increase their chances of survival and future reproduction (Magnhagen 1991). Conversely, individuals with less future reproductive potential often increase risk-taking behavior to optimize their current reproductive potential (Magnhagen 1990; Candolin 1998; Lafaille et al. 2010). They typically have a compromised ability to pay search costs (due to factors such as aging or decreasing time left in the season, as discussed above), but also have less reproduction potential to lose. As a result, they are often less sensitive to changes that could affect future reproductive opportunities. Interestingly, our results do not corroborate these studies. Females of all ages equally decreased their risk-taking behavior when they perceived predator cues.

One possible reason for our contrasting results may be that mating history, rather than female age, has a predominant effect on choosiness in *G. lineaticeps.* Though females differed in age, which has been shown to affect mate choosiness in other species

(Gray 1999; Stout et al. 2010), they did not differ in mating history, which as has also been shown to affect mate choosiness (Lickman et al. 1998). In our previous study, we found a significant effect of the interaction between female age and perceived male density: young females adjusted their choosiness based upon male density, whereas older females did not (Chapter 1). Clearly, female *G. lineaticeps* can adjust their choosiness based on age, but it is possible that mating history has a much larger effect than age. Indeed, it has been shown in a different species of field cricket, *G. pennsylvanicus,* that mating history, but not age, significantly influenced female choosiness (Judge et al. 2010; but see Matuz & Sakaluk 2008). Because all of our females were virgins, females of all three ages may have perceived that they had similar reproductive potential. Thus, females of all three age classes may have exhibited similar choosiness within the Predation and No Predation treatments.

Predation usually has major effects on fitness, but predation risk can vary on time scales from seconds to seasons (Lima & Dill 1990). Thus, investment in searching for and choosing mates should be influenced by how much reproductive potential the individual stands to lose as a result of predation (Candolin 1998; Lafaille et al. 2010) and the assessed level of predation risk (Lima & Dill 1990; Stankowich & Blumstein 2005). Assuming our differences in female age led to differences in future reproductive potential (Chapter 1), the result of this study indicates that females who perceive predation risk decrease their risk of death by being less choosy, regardless of future reproductive potential. Thus, predation should have a strong effect on the strength of sexual selection because of its effect on the choosiness of all females. When predators are common, and females are consequently less choosy, the strength of sexual selection on preferred male traits is decreased. When predators are absent, females can afford to increase their choosiness, thereby increasing selection for preferred male traits. Interestingly, this means the evolution of male mating signals can be influenced by predation both indirectly and directly. Male mating signals are often conspicuous and are known to increase the attraction of predators and the probability of predation (Endler 1991; Wagner 1996; Zuk & Kolluru 1998). Many male signaling tactics have thus evolved, directly in response to predation risk, that reduce the risk of death at the cost of mate attraction (Zuk & Kolluru 1998; Hughes et al. 2012). Additionally, predation may indirectly affect male signal evolution through its effect on female mating behavior (Johnson & Basolo 2003, this study). Consequently, predation risk may directly affect the evolution of important traits such as female mate choosiness or mating signals, as well as indirectly affect the strength and direction of sexual selection as it acts through plasticity in female mate choosiness.

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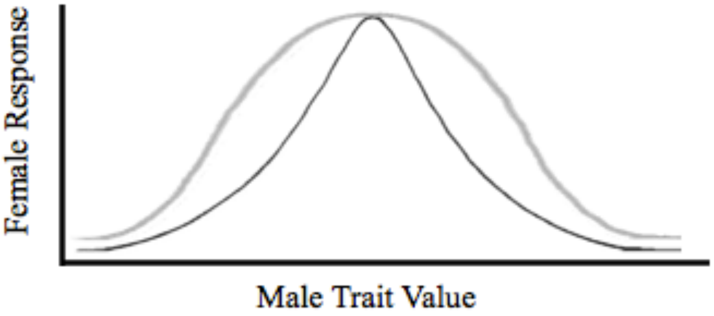

Figure 2.1. Theoretical representations of female choosiness. Females that are more choosy respond strongly only to a narrow range of male trait values (black line). Females that are less choosy respond strongly to a wider range of male trait values (grey line).

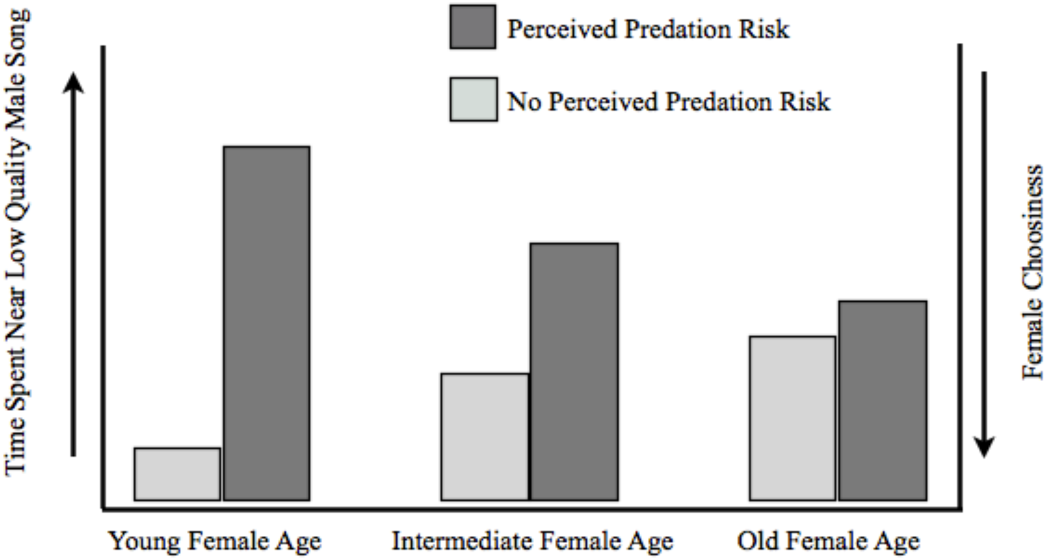

Figure 2.2. Predictions for female choosiness responses to the interaction of female age and perceived predation risk. We predicted that, when females perceive predation risk, choosiness decreases more for females of younger ages. This is exhibited by females spending more equivalent time near a low chirp rate song as they age.

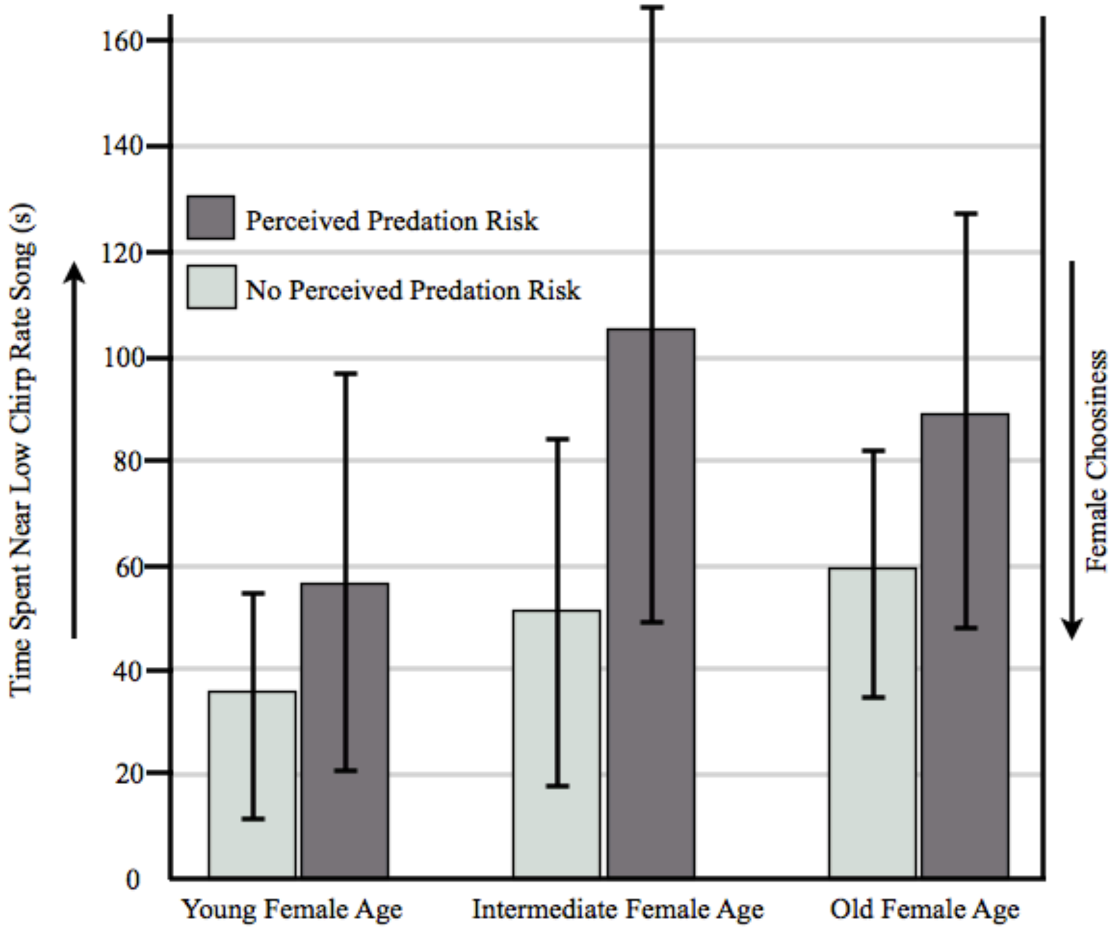

Figure 2.3. Mean time females spent near a low chirp rate song. The more time females spent near the song, the less choosy the females. Bars indicate 95% confidence intervals.

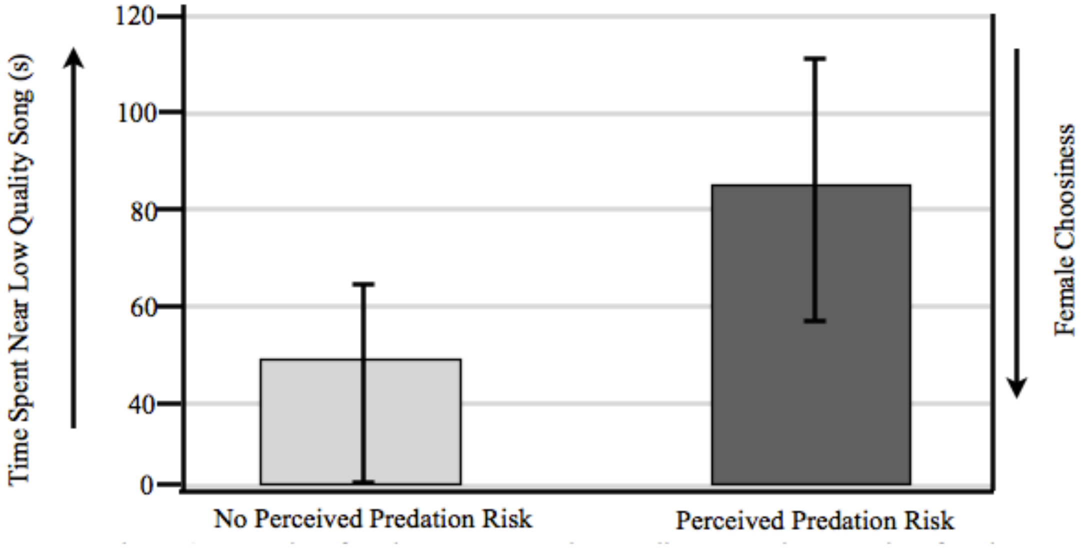

Figure 2.4. Mean time females spent near a low quality song. The more time females spent near the song, the less choosy the females. Females in the Perceived Predation Risk treatment spent significantly more time in the Choice Zone, and were therefore less choosy. Bars indicate 95% confidence intervals.