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COSTS OF FEMALE MATING BEHAVIOR IN THE VARIABLE FIELD CRICKET,  
*GRYLLUS LINEATICEPS*

by

Cassandra M. Martin

A DISSERTATION

Presented to the Faculty of  
The Graduate College of the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree of Doctor of Philosophy

Major: Biological Sciences  
(Ecology, Evolution, & Behavior)

Under the Supervision of Professor William E. Wagner, Jr.

Lincoln, Nebraska

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COSTS OF FEMALE MATING BEHAVIOR IN THE VARIABLE FIELD CRICKET,  
*GRYLLUS LINEATICEPS*

Cassandra M. Martin, Ph.D.

University of Nebraska, 2014

Advisor: William E. Wagner, Jr.

Female animals may risk predation by associating with males that have conspicuous mate attraction traits. The mate attraction song of male field crickets also attracts lethal parasitoid flies. Female crickets, which do not sing, may risk parasitism when associating with singing males. If parasitism risk is sufficiently high, it may affect the evolution of female mating behaviors. In this dissertation, I explore the interaction between the female variable field cricket, *Gryllus lineaticeps*, and the parasitoid fly, *Ormia ochracea*. To begin, I investigated whether female parasitism risk resulted from being near singing males. I found that females can become parasitized both by being near singing males when a fly arrives and by picking up previously deposited larvae when assessing males. Female crickets benefit from mating with males with high chirp rate song, but high chirp rate song attracts more flies. By caging male-female pairs above speakers broadcasting songs of different chirp rate, I found that females near high chirp rate song were more likely to be parasitized. Females with stronger preferences should thus incur higher costs. For fly parasitism to have a large effect on female mating behavior, it would need to affect fitness. I conducted a study to determine whether being parasitized affected female lifetime fecundity; I found that being parasitized reduced lifetime fecundity by over 90%. With parasitism occurring in a mating context, parasitism

being more likely when near preferred males, and parasitism reducing fitness, it should impact female cricket mating behavior. I conducted a study to determine whether females from a highly parasitized population discriminated between male songs of differing chirp rate; I found that they did not prefer high chirp rate song to intermediate chirp rate song, which is contrary to what would be expected based on benefits alone. This dissertation shows that the risk to female crickets of becoming parasitized is likely an association cost that has a large impact on female fitness and could affect female mating behavior.

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*Dedicated to my mother, Karen Marie Martin, who was not able to see me finish the dissertation and defense process, but so wanted to see it happen.*

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## INTRODUCTION TO THE DISSERTATION

Cassandra M. Martin

## PROBLEM

This dissertation explores the costs of mating behavior in female field crickets. Female animals can incur costs at every point in the mating process, from finding a mate to interacting with a mate to actually mating. Most of the costs of female mating behavior can be classified into three categories: search costs, mating costs, and association costs. Search costs are incurred when females actively seek out mates, thereby exposing themselves to predators and the environment and thereby diverting time and energy from other activities (reviewed in: Reynolds & Gross 1990). Mating costs are incurred during copulation and include: attracting predators, acquiring sexually transmitted diseases, and being injured by the mate (reviewed in: Arnqvist & Rowe 2005). Association costs are incurred when proximity to a male results in detrimental effects to the female. When males have conspicuous traits in order to attract mates, these traits can also attract predators (reviewed in: Burk 1982; Lima & Dill 1990; Sakaluk 1990; Magnhagen 1991; Zuk & Kolluru 1998; Haynes & Yeorgan 1999; Robinson & Hall 2002) or competitors (Andersson 1994). Females near males with conspicuous traits may incur increased predation risk (reviewed in: Lima & Dill 1990; Sakaluk 1990; Magnhagen 1991; Jennions & Petrie 1997; Hughes et al. 2012). Similarly, females may be at risk of injury if they are too near males engaged in male-male competition (Leboeuf & Mesnick 1991). This dissertation focuses on association costs, specifically predation-related association costs.

Association costs have the potential to affect the evolution of a variety of female mating behaviors. For example, when it is risky to associate with males with attractive traits, females are predicted to be less discriminating or even reverse their preferences

and prefer safer males (Magnhagen 1991; Jennions & Petrie 1997). There is some evidence that female choosiness can be affected by environmental factors such as predation risk. Most studies of this kind find predator-induced plasticity in female behavior; females in such studies reversed their preferences or became less discriminating in the presence of predators or predator cues (Forsgren 1992; Hedrick & Dill 1993; Godin & Briggs 1996; Gong & Gibson 1996; Gong 1997; Johnson & Basolo 2003; Bonachea & Ryan 2011). For instance, female sand gobies, *Pomatoschistus minutus*, spent less time with larger and more brightly colored males in the presence of a predator than in the absence of a predator (Forsgren 1992). Only a handful of studies have investigated the evolutionary consequences of predation on female preferences; in guppies, *Poecilia reticulata*, females from areas of high predation are less discriminating than females from areas of low predation (Houde & Endler 1990; Houde 1993; Endler & Houde 1995). These weaker preferences in areas of high predation may result from association costs; female guppies have a higher risk of predation when near brightly colored males than when near duller males (Pocklington & Dill 1995).

## STUDY SYSTEM

Investigating the effect of predation-related association costs on the evolution of female mating behavior requires a system in which male signals are known to attract predators and in which females are known to risk predation when near males. These criteria have only been established empirically in a small number of systems, one of which is field crickets. In most field cricket species, male crickets sing to attract females (Alexander 1961). However, singing males also attract the lethal phonotactic parasitoid



fly, *Ormia ochracea* (Cade 1975). Females do not sing and, thus, do not directly attract parasitoid flies; however, females are sometimes parasitized (Walker & Wineriter 1991; Adamo et al. 1995b). After landing near a male, *Ormia ochracea* forcefully expels early-stage larvae at its potential host (Cade 1975; Adamo et al. 1995b). Some larvae fall on the host and burrow into the cricket's body, while some larvae fall on the ground near the intended host and can potentially infect other crickets that later contact those larvae (Cade 1975; Walker & Wineriter 1991; Adamo et al. 1995b; Lehmann 2003). Therefore, females may be at risk of becoming directly parasitized if they are near a male when a fly arrives, and females may be at risk of becoming indirectly parasitized if they pick up previously deposited larvae when near a male who had earlier attracted flies. The risk of becoming parasitized while near a calling male is likely low because once a female decides to mate, she and the male retreat into his burrow where they are safer from being directly attacked by the adult parasitoid. However, the risk to females of picking up previously deposited larvae could be quite high. Females may traverse larvae laden areas while assessing the male and while leaving the male, and may even pick up larvae from the male himself.

Parasitism by *O. ochracea* is deadly. The parasitoid larvae burrow into the cricket, feed on its tissues, and emerge seven to ten days after infection (Walker & Wineriter 1991; Zuk et al. 1993; Adamo et al. 1995b). As the larvae emerge, they purge their gut contents inside the cricket; the cricket dies soon after from a combination of tissue damage and systemic poisoning (Adamo et al. 1995a). We know that being parasitized is costly for males and females. For both sexes, lifespan is affected: as crickets typically live for two to four weeks in the field (Simmons & Zuk 1994; Murray

& Cade 1995), being parasitized could reduce their lifespan, quite severely if the cricket was young when parasitized. With a shorter lifespan, a cricket would have less time to reproduce and would likely have lower fitness. There is substantial evidence that being parasitized affects reproduction in males: parasitized males call less (Zuk et al. 1995; Kolluru 1999; Orozco & Bertram 2004; Beckers & Wagner 2011) and show reduced courtship activity (Adamo et al. 1995a); therefore, they would attract and successfully mate with fewer females. The cumulative effects of parasitism have been quantified in the bushcricket species *Poecilimon marianne* parasitized by the fly *Therobia leonidei*: parasitized males lost 42% of their potential lifetime reproductive success compared to non-parasitized males (Lehmann & Lehmann 2006). There is less evidence that being parasitized affects reproduction in females. However, we do know that parasitized females lay substantially fewer eggs than non-parasitized females in three *Gryllus* field cricket species (Adamo et al. 1995a). In addition, recent evidence shows that parasitized females become less discriminating (Beckers & Wagner 2013), and thus may forego the fecundity benefits of being choosy (Wagner & Harper 2003; Tolle & Wagner 2011).

Because the costs of fly parasitism are significant (early death and decreased reproduction while still alive), parasitism risk should affect the evolution of mating behavior. In fact, selection from fly parasitism has been so strong in one species of field cricket that many males have lost the ability to call; in a heavily parasitized population of *Teleogryllus oceanicus* in Hawaii, a mutation, known as flatwing, which prevents males from singing, has arisen and increased to high frequency over just a few years (Zuk et al. 2006). Less drastic changes are seen in field cricket species, *Gryllus rubens*; males call less (Velez & Brockmann 2006b) and females are less responsive to male song (Velez &

Brockmann 2006a) in autumn when flies are present than in spring when flies are not present.

## **SUMMARY OF MY RESEARCH**

In this dissertation research, I explore the interaction between the female variable field cricket, *Gryllus lineaticeps*, and the parasitoid fly, *Ormia ochracea*. My overarching interest was in studying possible effects of parasitism risk on female cricket mating behavior. In some populations of the variable field cricket, *G. lineaticeps*, females prefer male calls with higher chirp rates (Wagner 1996). Females can benefit from this preference: nutritionally stressed females receive direct benefits from mating with males with calls of higher chirp rate (Wagner & Harper 2003), at least in some environments (Tolle & Wagner 2011). Just as females often prefer male calls with higher chirp rates, *Ormia ochracea* also preferentially orients to calls with higher chirp rates (Wagner 1996; Wagner & Basolo 2007a). Because males with higher chirp rate calls attract more flies, females should have a higher risk of parasitism when near these males. Females with stronger preferences should incur more costs than females with weaker preferences, potentially leading to population level changes in female preferences.

Before I could study effects of parasitism on mating behavior, it needed to be established that the parasitism risk for female crickets was an association cost. To do this, I needed to determine whether the risk of parasitism for female crickets resulted from approaching and being near singing male crickets. I conducted studies that examined whether females could become parasitized when near singing males, whether the risk of parasitism differed for females near males with different song characters, and whether

females could become parasitized by picking up previously deposited larvae. For fly parasitism to have a large effect on female mating behavior, it would need to have fitness costs; I conducted a study to determine whether being parasitized affected female lifetime fecundity. After establishing the previously unknown background information, I was able to investigate whether the risk of parasitism affects female mating behavior. I conducted a study to determine whether females from a highly parasitized population discriminated between male songs differing in chirp rate.

In chapter one, I ask whether female crickets are at risk of parasitism when near male song and whether some male song types are more risky to associate with than others. It is already known that females of other field cricket species are parasitized (Walker & Wineriter 1991; Adamo et al. 1995b), and that the flies that co-occur with *G. lineaticeps* prefer higher chirp rate song to lower chirp rate song (Wagner 1996; Wagner & Basolo 2007a). However, it is not known when and how females become parasitized and whether crickets in association with different songs of different chirp rate actually have a differential parasitism risk. To examine this, I conducted a field experiment in which I caged pairs of male and female crickets above speakers broadcasting songs of different chirp rate, and then monitored the crickets for parasitism. I found that female crickets were parasitized when in association with male song, suggesting that at least part of their parasitism risk stems from being near singing males in a mating context. I also found that females were much more likely to become parasitized when near high chirp rate song. Females with stronger chirp rate preferences would have a higher parasitism risk, and thus, parasitism risk may affect the strength and direction of female preferences.

In chapter two, I ask whether female crickets can become parasitized by picking up previously deposited larvae, and if so, what is the duration of that risk. Female flies deposit some larvae on the ground around an acoustically-located host that could later infect collateral hosts (Cade 1975; Walker & Wineriter 1991; Adamo et al. 1995b). *O. ochracea* larvae can live for at least eight hours (Beckers et al. 2011); however, we did not know if they were still infectious. I conducted a study to determine how long fly larvae remain infectious after being deposited. I prepared dishes with previously deposited larvae and exposed females to them zero, two, and six hours later. I found that the risk of becoming parasitized did not diminish with time, and that approximately 20% of all females that traversed the minefield of larvae became parasitized. Females can become parasitized by when near a singing male both by being directly parasitized and by picking up previously deposited larvae. The risk of picking up previously deposited larvae is quite high and does not diminish throughout the nightly mating period.

In chapter three, I ask whether being parasitized reduces lifetime fecundity and whether any reduction differs between becoming parasitized at a younger and an older age. If a female cricket becomes parasitized when she is near the end of her reproductive lifespan, then parasitism, although deadly, may actually not be very costly in terms of fitness. However, if a young female becomes parasitized, she could lose a substantial amount of her lifetime reproductive success. I infected and sham-infected females with fly larvae at a younger and an older age, and I then monitored their egg output. Infected females laid fewer eggs than sham-infected females, and I found a larger impact on the fecundities of younger females than older females. Not only does fly predation lead to death, but it substantially reduces female fecundity while still alive.

In chapter four, I ask whether mate choice in female crickets has been affected by the risk of parasitism. If it is more risky to be near high chirp rate song, female preferences for high chirp rate song may be reduced. I performed mate choice tests where I presented females from a highly parasitized population with pairs of songs varying only in chirp rate. I found that females discriminated against low chirp rate song but did not discriminate between intermediate and high chirp rate song. This may indicate a compromise between the benefits of choosing higher chirp rate males and the costs of becoming parasitized.

In this dissertation, I establish that the risk to female crickets of becoming parasitized is likely the result of an association cost that has a large impact on female fitness and could affect female mating behavior. I found that females can become parasitized by both being near singing males and by picking up previously deposited larvae. Additionally, I found that females near high chirp rate song, which is preferred by the flies and by female crickets from some populations (Wagner 1996; Wagner & Basolo 2007a), are more likely to be parasitized. I also found that there is a fitness cost to females of becoming parasitized beyond a reduced reproductive lifespan. With parasitism occurring in a mating context, parasitism being more likely when near preferred males, and parasitism reducing fitness, it should impact female cricket mating behavior. I found that females from a highly parasitized population did not prefer high chirp rate song to intermediate chirp rate song as females from some non-parasitized populations do (Wagner 1996; Wagner & Basolo 2007b), which suggests that parasitism has affected mate choice to some degree. Studies comparing mating behaviors of female crickets from parasitized and non-parasitized populations could provide more solid evidence that the

risk of fly parasitism for females, a likely association cost, has affected the evolution of female mating behavior.

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**CHAPTER 1****FEMALE FIELD CRICKETS INCUR INCREASED PARASITISM RISK WHEN  
NEAR PREFERRED SONG**

Cassandra M. Martin and William E. Wagner, Jr.

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## ABSTRACT

Female animals often prefer males with conspicuous traits because these males provide direct or indirect benefits. Conspicuous male traits, however, can attract predators. This not only increases the risk of predation for conspicuous males but also for the females that prefer them. In the variable field cricket, *Gryllus lineaticeps*, males that produce preferred song types provide females with greater material benefits, but they are also more likely to attract lethal parasitoid flies. First, we conducted a field experiment that tested the hypothesis that females have a greater risk of fly parasitism when in association with preferred high chirp rate males. Females were nearly twice as likely to be parasitized when caged with high chirp rate song than when caged with low chirp rate song. Females may thus be forced to trade off the quality of the benefits they receive from mating with preferred males and the risk of being killed by a predator when near these males. Second, we assessed female parasitism rates in a natural population. Up to 6% of the females were parasitized in field samples. Because the females we collected could have become parasitized had they not been collected, this provides a minimum estimate of the female parasitism rate in the field. In a laboratory study, we found no difference in the proportion of time parasitized and non-parasitized females spent hiding under shelters; thus, differences in activity patterns do not appear to have biased our estimate of female parasitism rates. Overall, our results suggest that female association costs have the potential to shape the evolution of female mating preferences.

## INTRODUCTION

Male animals often express conspicuous traits that increase their probability of attracting females, but these traits can also increase their probability of attracting predators (Cade 1975; Tuttle & Ryan 1981; Sakaluk & Belwood 1984; Slagsvold et al. 1995; Zuk & Kolluru 1998; Bernal et al. 2006). Males of many species may thus be forced to trade off the benefits of attracting mates and the risks of attracting predators. Females often prefer males with these conspicuous traits because these males provide material benefits that increase female fitness or genetic benefits that increase offspring fitness (Andersson 1994). Associating with conspicuous males, however, might increase a female's risk of predation because of conspicuous males' higher probability of attracting predators. As a result, females, like males, may be forced to trade off mating benefits and predation costs. Association costs have the potential to have a powerful effect on sexual selection. Association costs may limit the expression of female preferences or favor females that select mates based on alternative, less risky traits. Because of these effects on female preferences, association costs may also change the nature of sexual selection on male traits. Few laboratory studies have shown that females might incur association costs (Pocklington & Dill 1995), and little is known about such costs in the field.

Field crickets provide a striking example of the predation costs of male signals. Males of some species are parasitized by the parasitoid fly, *Ormia ochracea* (Cade 1975; Walker & Wineriter 1991; Zuk et al. 1993). These flies locate their hosts by orienting to male song and then deposit larvae on and around males (Cade 1975). Larvae landing around males will latch onto anything moving by them (Cade 1975), and in a related

parasitoid, *Homotrixia alleni*, the larvae can live for up to two hours outside of a host (Allen et al. 1999). Thus, in addition to being directly parasitized, crickets are also at risk of becoming parasitized by previously deposited larvae. Once the larvae contact a cricket, they enter the cricket where they feed and grow. The larvae emerge seven to twelve days later to pupate, and the cricket dies shortly thereafter (Cade 1975; Walker & Wineriter 1991; Zuk et al. 1993). Previous studies have shown that the flies preferentially orient to the same song types that female crickets prefer (Wagner 1996; Gray & Cade 1999; Lehmann et al. 2001; Wagner & Basolo 2007a). As a result, males that produce song types preferred by females may have a higher risk of fly parasitism (Zuk et al. 1998).

Although female crickets do not sing, they are occasionally parasitized (Walker & Wineriter 1991; Adamo et al. 1995b). Nothing is known about the context of female parasitism, but it presumably occurs when females are in association with singing males. In many field crickets, males sing from just outside the entrance to a burrow (Alexander 1961). When a female approaches a singing male, she might pick up previously deposited larvae from the ground. In addition, once the female is near the male, the two directly interact for a short time while the male produces both calling and courtship songs (Alexander 1961). During this time, the female might be indirectly parasitized, picking up previously deposited larvae from the ground or from the male, or be directly parasitized by a recently attracted fly. If a female decides to mate, the pair then retreats into the male's burrow where the risk of fly parasitism is likely much lower.

There is substantial evidence that being parasitized is costly for males. First, parasitized crickets die within seven to twelve days of being parasitized (Cade 1975; Walker & Wineriter 1991; Zuk et al. 1993). Males typically live for two to four weeks as

adults in the field (Simmons & Zuk 1994; Murray & Cade 1995), so being parasitized may often significantly reduce a male's lifespan. Second, male reproduction while still alive can be severely reduced: parasitized males sing less (Zuk et al. 1995) and would therefore attract fewer mates, show reduced courtship activity (Adamo et al. 1995a), and have reduced reproductive effort (Kolluru et al. 2002). As a result of these types of effects, Lehmann and Lehmann (2006) calculated that male bushcrickets, *Poecilimon mariannae*, parasitized by *Therobia leonidei* lost 42% of their potential lifetime reproductive success compared to non-parasitized males. Being parasitized is also likely to be costly for females. Like males, parasitized females have a reduced lifespan. In addition, female egg laying precipitously declines within five days of being parasitized in several species (Adamo et al. 1995a). The costs of being parasitized are likely to be very high for younger males and females that are just beginning to reproduce, but even older individuals may incur costs.

In the variable field cricket, *Gryllus lineaticeps*, males that produce higher chirp rates are more likely to attract both conspecific females and parasitoid flies (Wagner 1996; Wagner & Basolo 2007a). Females receive fecundity benefits from mating with males with higher chirp rates (Wagner & Harper 2003), but because these males are more likely to attract parasitoid flies, females may not only risk fly parasitism when in association with males, but also may incur a greater risk when in association with preferred males. We used a field experiment to test the hypothesis that females in association with higher chirp rate song incur a higher risk of fly parasitism. We then assessed the parasitism rate of female crickets in the field and used a laboratory infection

study to assess whether our estimate of female parasitism rate was biased by differences in the activity patterns of parasitized and non-parasitized females.

## METHODS

### *Parasitism Risk Experiment*

All *Gryllus lineaticeps* used in this experiment were third- or fourth-generation lab-reared offspring of field-collected females from Rancho Sierra Vista, Santa Monica Mountains National Recreation Area near Thousand Oaks, California, USA. This population is known to be parasitized by *Ormia ochracea* (Wagner & Basolo 2007a). Matings between individuals of known ancestry were arranged to minimize inbreeding in our colony. Full sibling families were reared in 25 x 15 x 17 cm clear plastic containers that were outfitted with egg carton shelters, a paper towel substrate, vermiculite containers for oviposition, water vials with cotton plugs and ad libitum Purina Cat Chow®. At the penultimate stadium, individuals were moved to 15 x 8 x 11 cm clear plastic individual containers that were outfitted with shelter, substrate, water and food. In the laboratory in Lincoln, Nebraska, USA, crickets were maintained on a 14:10 hour light:dark cycle at  $23 \pm 2^\circ\text{C}$ .

To test whether crickets in association with high chirp rate song have a greater parasitism risk than those in association with low chirp rate song, lab-reared crickets were transported to the field site, Rancho Sierra Vista, where we placed male-female pairs in cages above speakers broadcasting either high or low chirp rate *G. lineaticeps* calling song. The synthetic songs we used are described in Wagner and Basolo (2007b). In brief, a natural pulse was digitized and used to create a chirp that contained eight pulses (chirp



duration = 120 ms). This chirp was then used to create a high chirp rate song (4.2 chirps/s) and a low chirp rate song (1.8 chirps/s), each of which was recorded to a compact disc. In the field, Pyramid MDC-6 waterproof speakers (13.35 cm diameter) were buried flush with the ground and oriented with the speaker cone facing upward. We placed cylindrical cages (15.2 cm diameter, 10.2 cm height) on top of the speakers. The cages were constructed of size five 24-gauge galvanized wire mesh; the openings in the mesh were large enough for flies to pass through but small enough to contain the crickets. The bottom of the cage was covered with speaker grill cloth to prevent parasitoid fly larvae from falling through onto the speaker. We set up ten of these cages on top of speakers; they were evenly spaced 5 m apart in two rows of five. Song was broadcast through the speakers using Coby CX-CD567 and Coby CX-CD587 weather-resistant personal compact disc players and Sonic Impact Technologies 5066 portable 15 W class-T amplifiers. For each pair of speakers, one broadcast the high chirp rate song and the other broadcast the low chirp rate song. The songs were switched between speakers between nights to prevent biases based on speaker location.

Eighty trials were run from 11-21 August 2007 in the field at Rancho Sierra Vista. All crickets used in the experiment were between 5 and 21 days post adult eclosion, lab-reared, and housed away from parasitoid flies. Thus, all crickets were known to be non-parasitized at the start of the experiment. Depending upon the number of crickets of the correct age and sex that were available, we set up between six and ten cages per night. Prior to sunset, we placed a female and a muted male in each of the cages positioned above the speakers; we muted males by sealing their forewings with beeswax so that we could control chirp rate. Song was then broadcast for 30 minutes, beginning at sunset

(between 2040-2050), at an amplitude of 90-92 dB SPL (re: 20 $\mu$ Pa) measured 35 cm above the speaker. The cages were checked for parasitoid flies 10, 20 and 30 minutes following the start of a trial using a headlamp. The song amplitudes of males are typically 67-79 dB SPL (re: 20 $\mu$ Pa) at 30 cm (Wagner unpublished data), and the male-female pair would only spend a few minutes together above ground while the male is singing. However, in order to complete the experiment in a practical amount of time, we purposely exaggerated the absolute parasitism risk by forcing the crickets to associate with a high amplitude song (to attract a sufficient number of flies) for a longer period (to allow sufficient time for parasitism to occur). While absolute parasitism rates for crickets in the experiment were unnaturally high, we were interested in the relative difference in risk for crickets in association with the two chirp rates. We discuss the potential consequences of this experimental design choice in the discussion.

After a trial was finished, the crickets were returned to their individual containers and monitored for parasitoid pupae for 15 days (the emergence range for *O. ochracea* pupae from *G. lineaticeps* for this experiment was 8-12 days:  $X \pm SE = 9.7 \pm 0.1$  days,  $N = 82$  crickets). Any cricket that died before 15 days was dissected to determine its parasitism status. Crickets remaining alive at the end of monitoring were frozen and later examined for parasitoid larvae by dissection to ensure that 15 days of monitoring was a sufficient criterion for detecting parasitism; none of these dissected crickets were parasitized. Two males escaped the cage during a trial and two males were lost before their parasitism status could be determined; data from those males were excluded, resulting in 39 high chirp rate males and 37 low chirp rate males. Females paired with the males that escaped during a trial were not included in the analysis because the absence of

the male may have changed their risk of parasitism; however, females paired with males that were lost subsequent to the completion of a trial were included in the analysis, resulting in 40 high chirp rate females and 38 low chirp rate females.

### *Parasitism Rates*

We collected male and female *G. lineaticeps* from Rancho Sierra Vista to determine parasitism rates in the field. All crickets were collected by visually searching with a headlamp in areas with low or no vegetation (crickets are difficult to observe and collect in vegetation). In order to separate crickets collected before and during the period when flies were active, we checked for fly activity each night by observing whether flies oriented to male song. We did this by broadcasting synthetic male song from compact disc played on either Coby CX-CD567, Coby CX-CD587 or Sony CD Walkman D-EJ011 personal compact disc players and Saul Mineroff SME-AFS Portable Field Speakers at 80-90 dB SPL (re: 20 $\mu$ Pa) at 30 cm from speaker. In 2007, we began sampling for flies on 15 July and flies were observed at that first broadcast. In 2008, we began sampling for flies on 10 July and sampled a minimum of twice per week; flies were first observed on 15 August and did not reach appreciable numbers (greater than two per broadcast) until 30 August.

In 2007, male and female crickets were collected from 15 July to 22 August after the parasitoid flies had already become active. In 2008, female crickets were collected from 10 July to 9 August, before the flies became active, and from 15 August to 14 September, after the flies became active. No males were collected in 2008 because we were interested in focusing on female parasitism rates. In both years, crickets were

collected sporadically within each time period, with the average time between collections being two days. Field collected crickets were brought to an indoor space away from flies and housed in individual plastic containers with shelter, substrate, water and food. We checked the containers daily for the presence of parasitoid pupae for a minimum of 15 days post collection. If a cricket died before 15 days without the appearance of pupae, it was dissected to determine parasitism status.

#### *Activity Patterns Experiment*

Differences in the activity patterns of parasitized and non-parasitized females might have biased our female parasitism estimates (e.g., parasitized females might spend more or less time exposed than non-parasitized females). In order to assess the importance of such a bias, we examined the activity of parasitized and non-parasitized female *G. lineaticeps* in an arena in the laboratory. The crickets used in this experiment were second-generation lab-reared offspring from field-caught females from Rancho Sierra Vista. To produce parasitized crickets, we transported gravid *O. ochracea* females from Rancho Sierra Vista to the laboratory in Lincoln, Nebraska. Six or fewer flies were housed in each clear plastic container (25 x 15 x 17 cm). Each container had shredded paper towel for substrate, a dish with sugar cubes and cotton that was wetted with sugar water, and another dish with natural applesauce. We then hand-infected some females by depositing larvae on the soft tissue in the space between their pronotum and wings using a dissecting probe; we attempted to deposit two larvae per cricket, but there was some variation in the number of larvae that emerged from the experimentally infected females ( $X \pm SE = 2.1 \pm 0.3$  larvae,  $N = 10$  crickets). The non-parasitized females were sham-

infected by handling them in the same manner as the hand-infected females, except we used a clean probe instead of one with larvae on it. The females were housed in individual containers with shelter, substrate, water and food in an acoustically isolated room on a 14:10 hour light:dark cycle at  $23 \pm 2$  °C.

Female activity patterns were assessed in a 3.65 x 1.2 x 0.65 m plywood arena. The inner walls of the arena were covered with black plastic to prevent females from climbing out of the arena, and the substrate consisted of a thin layer of sand. Twelve egg carton shelters (10 x 10 cm) were placed in two rows of six inside the arena; the two rows were 50 cm apart and the shelters in each row were 45 cm apart. Small plastic Petri dishes, with three pieces of cat food in each, were placed equidistant between adjacent shelters in each row to encourage the female crickets to leave the shelters and forage, as they would naturally have to leave shelter to find food. Three clip-on desk lamps with red bulbs illuminated the arena.

All crickets were tested two and six days post infection (or sham-infection). Previous work indicated that parasitism does not affect behavior or reproduction until three to five days post infection (Adamo et al. 1995a), so we choose a time earlier in infection where the parasitoid should have less of an effect on the host, and a time later in infection where the parasitoid should have more of an effect. Six days was chosen as the later day in order to represent all of the infected crickets as some crickets die as early as seven to eight days after being parasitized and thus would not have been able to participate in the experiment. Prior to the first test at two days, each cricket was marked using a unique combination of colored dots of correction fluid placed on the dorsal surface of the thorax. Three parasitized and three non-parasitized females were tested in

each trial. The six females were placed in the arena with the fluorescent room lights on for 10-12 hours prior to the start of observations. No song was broadcast during this period of simulated daylight. The room lights were then turned off and song was broadcast to simulate nighttime conditions. The high chirp rate song used to assess the effect of chirp rate on parasitism risk was broadcast at 60 dB SPL (re: 20  $\mu$ Pa) at 50 cm from speakers located on the ground outside each of the narrow ends of the arena. The song was broadcast from compact disc on a Sony CD Walkman D-EJ011 personal compact disc player connected to a Sonic Impact Technologies 5065 Gen2 portable 15 W class-T digital amplifier and Pyramid MDC-6 waterproof speakers (13.35 cm diameter). The broadcasts were designed to provide incentives for the female crickets to move around in the arena to search for singing males, as would occur under natural conditions. The crickets were acclimated to these nighttime conditions for one half hour before beginning the three-hour observation period. During this three-hour period, the location of each cricket was noted every 10 minutes (in the open or hiding beneath a shelter) by spot-checking with a headlamp (this was necessary to observe the unique markings on the thoraxes of the females). Each trial thus yielded 19 samples of female activity (beneath a shelter or not beneath a shelter).

A total of 12 parasitized and 12 non-parasitized females were tested two and six days following infection/sham-infection between 27 September and 6 October 2008. Two of the infected females, however, did not yield parasitoid pupae. Because we could not determine parasitism status until parasitoid pupae emerged, those two crickets were run in the experiment, but they were not included in the analysis. The resulting sample size was thus 10 parasitized and 12 non-parasitized females.

## RESULTS

### *Parasitism Risk Experiment*

Parasitoid flies were more likely to be observed in the high chirp rate (HCR) cages than in the low chirp rate (LCR) cages (HCR: 35/40, LCR: 24/38; Fisher's exact test:  $P = 0.017$ ). Because flies were more likely to be attracted to the higher chirp rate song, cages in the high chirp rate treatment were more likely to contain at least one parasitized cricket than cages in the low chirp rate treatment (HCR: 34/39, LCR: 20/37; Fisher's exact test:  $P = 0.002$ ). There was a tendency for males in the high chirp rate treatment to be parasitized more frequently than males in the low chirp rate treatment (HCR: 24/39, LCR: 16/37; Fisher's exact test:  $P = 0.168$ , Fig. 1.1 A). Females in the high chirp rate treatment, however, were significantly more likely to be parasitized than females in the low chirp rate treatment (HCR: 29/40, LCR: 15/38; Fisher's exact test:  $P = 0.006$ , Fig. 1.1 B); the parasitism risk for females in the high chirp rate treatment was 1.8 times greater than that for females in the low chirp rate treatment.

### *Parasitism Rates*

In the 2007 collection, which occurred during an unknown period of time after the start of fly activity, approximately 1% of female crickets collected were parasitized (1 of 104) and 59.1% of male crickets collected were parasitized (13 of 22). The disparity between the number of females and males collected was probably due to the lower likelihood of encountering males using visual search methods; males remain near their burrows during nighttime hours whereas females move around actively searching for males. In the 2008 collection, no females were parasitized before the flies were observed

(0 of 50), while 6.1% of females were parasitized after the flies were observed (3 of 49). No males were collected in 2008.

#### *Activity Patterns Experiment*

Parasitized and non-parasitized female crickets did not differ in the number of samples in which they were hidden under shelters, either two days following parasitism (Mann-Whitney  $U$  test:  $z_{20} = 1.051$ ,  $P = 0.293$ , Fig. 1.2 A) or six days following parasitism (Mann-Whitney  $U$  test:  $z_{20} = 0.840$ ,  $P = 0.401$ , Fig. 1.2 B). Furthermore, neither parasitized females (Wilcoxon matched-pairs signed-rank test:  $z_8 = 0.255$ ,  $P = 0.799$ ) nor non-parasitized females (Wilcoxon matched-pairs signed-rank test:  $z_{10} = 0.237$ ,  $P = 0.813$ ) showed changes in their shelter use from day two to day six.

## **DISCUSSION**

In the variable field cricket, *Gryllus lineaticeps*, females from at least some populations prefer males that produce higher chirp rate song (Wagner 1996), and males with higher chirp rates appear to transfer seminal fluid products to females that enhance female fecundity (Wagner & Harper 2003). Our results, however, suggest that to obtain these benefits, females in populations parasitized by *Ormia ochracea* may have to incur a greater risk of fly parasitism. In our field experiment, females in association with high chirp rate song were 1.8 times more likely to be parasitized than females in association with low chirp rate song. This greater risk is at least partially because higher chirp rates are more likely to attract flies, as was found in this and other studies (Wagner 1996; Wagner & Basolo 2007a). Because the inevitable result of fly parasitism is death, the cost



for females is potentially quite severe, particularly for young females that may lose a substantial proportion of their reproductive lifespan if they are parasitized. This cost is magnified by the fact that female egg laying drops sharply between three and five days post infection (Adamo et al. 1995a). Field crickets typically live for two to four weeks as adults in the field (Simmons & Zuk 1994; Murray & Cade 1995), and rather than periodically laying discrete clutches of eggs, females lay a small number of eggs each day they remain alive. If a female is parasitized at a young age, her reproductive lifespan will be reduced from a few weeks to a few days, which should substantially reduce her lifetime reproductive success. Because of the cost of fly parasitism, and because of the higher risk that appears to result from being near high chirp rate song, the evolution of female song preferences in this species may thus be affected not only by the benefits of mating with high chirp rate males, but also by the risk of fly parasitism that results from associating with them.

Surprisingly, males in the high chirp rate treatment were not parasitized significantly more often than males in the low chirp rate treatment, despite the fact that the high chirp rate cages were significantly more likely to attract flies. There are several possible explanations for this puzzling result. While the difference was not statistically significant, there was a tendency for high chirp rate males to be parasitized more frequently than low chirp rate males. A larger sample size might have allowed us to detect a difference. Because females were present for the entire duration of fly exposure, the overall male parasitism rate may have been lower making it more difficult for us to detect relative differences between the chirp rate treatments. For instance, it is possible that the flies use cues other than sound to choose hosts once the general location of the

host is established; the females, which are on average larger than males, might be easier or more profitable targets for the flies. Additionally, females might have been more active in the cages, resulting in a higher encounter rate with flies and/or larvae deposited on the substrate. And finally, males are likely to have evolved more effective anti-parasitoid tactics than females, as males are likely under stronger selection from fly parasitism.

Our experimental design purposely exaggerated the absolute risk of fly parasitism for females so that we could examine differences in relative risk using a practical number of replicates. The primary exaggerations were broadcasting male song at a high amplitude and forcing the females to remain in association with males while above ground for an extended period of time. For these reasons, the absolute parasitism risk for females and males is certainly much lower than our experiment would suggest. It is also possible that these methodological choices biased our estimates of the relative risk of associating with high and low chirp rate song. While it seems unlikely that the use of high amplitude song could cause a difference in relative risk that is otherwise not present, the real relative risk could be lower or higher than we found in our experiment depending upon whether the flies show either a lesser or greater chirp rate discrimination at high amplitudes. Ramsauer and Robert (2000) found that the flies would respond to simulated *G. rubens* song with carrier frequencies not naturally present in their songs when presented at high amplitudes, suggesting that the flies may actually be less discriminating at high amplitudes. Whether a long duration of association could bias estimates of relative risk depends, in part, on whether females that approach high and low chirp rate males spend different amounts of time above ground before entering the male's burrow

where the female's risk is likely much reduced. If females that approach high chirp rate males and females that approach low chirp rate males spend similar amounts of time above ground before entering the burrow, the natural difference in relative risk should be similar. If, however, females take longer to enter the burrows of low chirp rate males, the natural difference in relative risk may be less than our results suggest; taking longer to enter may increase the risk that a fly will arrive before the female enters the burrow and may also increase the risk the female will pick up previously deposited larvae. Such a difference in behavior should be disfavored by selection. If there is a risk of fly parasitism, females should only approach males with which they are interested in mating, and they should quickly enter the male's burrow. Once in the burrow, they can assess non-calling song traits (e.g., courtship song, tactile signals and any chemical signals) with less risk. In addition to exaggerating some conditions, we chose to base all song characteristics (chirp duration, dominant frequency, etc.) except chirp rate on the average value for our population for both the high and low chirp rate stimuli. This could create issues for generalizing the results; for instance, perhaps the flies would respond differently if we used long chirp durations instead of average chirp durations.

In field samples collected during periods of fly activity, 1 and 6% of the females were parasitized. In related species attacked by *O. ochracea*, Walker and Wineriter (1991) found that approximately 10% of *G. rubens* and 10% of *G. firmus* females collected by systematic search were parasitized, and Adamo et al. (1995b) found that 3.2% of *G. integer* females that responded to male song broadcasts were parasitized. These studies and ours likely underestimate the actual parasitism rate for females. First, females do not become sexually mature until approximately seven days following their

final molt, and as a result, some of the females collected might not have been sexually mature, and thus might not have had opportunities to become parasitized. Second, the non-parasitized females that were sexually mature likely had a non-zero probability of later becoming parasitized had they not been collected. And third, it is possible that estimates of female parasitism rates could be biased by unequal probabilities of encountering parasitized and non-parasitized females. This could occur because parasitized females die at a faster rate and thus are less likely to be encountered and/or because parasitized and non-parasitized females differ in their activity patterns. For example, once parasitized, females might spend less time moving around above ground in search of food or mates, which could make them less likely to be collected using a visual search method. In our activity patterns experiment, we found that parasitized females did not hide more often than non-parasitized females, suggesting that differences in female activity probably did not substantially bias our parasitism rate estimates in *G. lineaticeps*. We did not, however, examine female activity in the later stages of parasitism (> 6 days post infection), which could affect the probability of parasitized females being represented in field samples as activity could change very late in parasitism.

Fly parasitism appears to have affected the evolution of male mating behavior in a number of species (Cade & Wyatt 1984; Zuk et al. 1993; Kolluru 1999; Bertram et al. 2004; Velez & Brockmann 2006b; Zuk et al. 2006). Whether the risk of fly parasitism for females is, or has been, sufficiently high enough to affect the evolution of female mating behavior is not known. Studies of a variety of organisms suggest that directional natural selection is typically weak (Endler 1986; Kingsolver et al. 2001). Even small effects on fitness, however, can result in large evolutionary changes given the cumulative effect of

selection over multiple generations. Given the relatively large difference in female risk when in association with high and low chirp rate males in *G. lineaticeps*, selection may be sufficiently strong to favor female behaviors that reduce the risk of fly parasitism, such as weaker preferences for high chirp rate males. In addition, the relatively low parasitism rates of females in nature might be a consequence of effective anti-parasitoid tactics that have already evolved, such as mating during times when the flies are less active (Cade et al. 1996), mating less frequently and/or choosing less risky males. For instance, in a single parasitized population of *G. rubens*, Velez and Brockman (2006a) found that autumn females, which experience fly parasitism, were less responsive to male song than spring females, which do not experience fly parasitism. However, comparative studies of parasitized and non-parasitized populations will be necessary to determine if the risk of fly parasitism has affected the evolution of female mating behavior, and whether the greater risk of associating with high chirp rate males has affected the evolution of female mating preferences.

Little is known about the predation risk that female animals incur from associating with males with more and less preferred traits, despite the importance of such costs for the evolution of female mating preferences. Choosy females, however, may risk predation in any species where preferred males are more conspicuous and likely to attract predators. Mate choice may thus often require a compromise between the benefits of mating with more preferred males and the lower risk of predation that results from mating with less preferred males. Guppies, *Poecilia reticulata*, are one of the only animals for which data are available on female association costs and the evolutionary consequences of these costs. Controlled laboratory experiments suggest that female guppies have a

greater risk of predation by a piscivorous cichlid when near more colorful males (Pocklington & Dill 1995), and female guppies from populations with a higher risk of predation have weaker preferences for conspicuously colored males (Endler & Houde 1995). Indirect evidence suggests that predation has had important effects on the evolution of female preferences in a variety of animals. For example, females in many species change their preferences when the perceived risk of predation is high (Forsgren 1992; Hedrick & Dill 1993; Godin & Briggs 1996; Gong & Gibson 1996; Johnson & Basolo 2003; Kim et al. 2007). Though these studies do not directly demonstrate the costs to females of being near conspicuous males, their results are consistent with an effect of these association costs on the evolution of female preferences. Because costs of female preferences can have profound effects on the nature and direction of sexual selection, more studies are needed to examine the existence of these costs in other taxa, as well as the evolutionary consequences of these costs in this species and other taxa.

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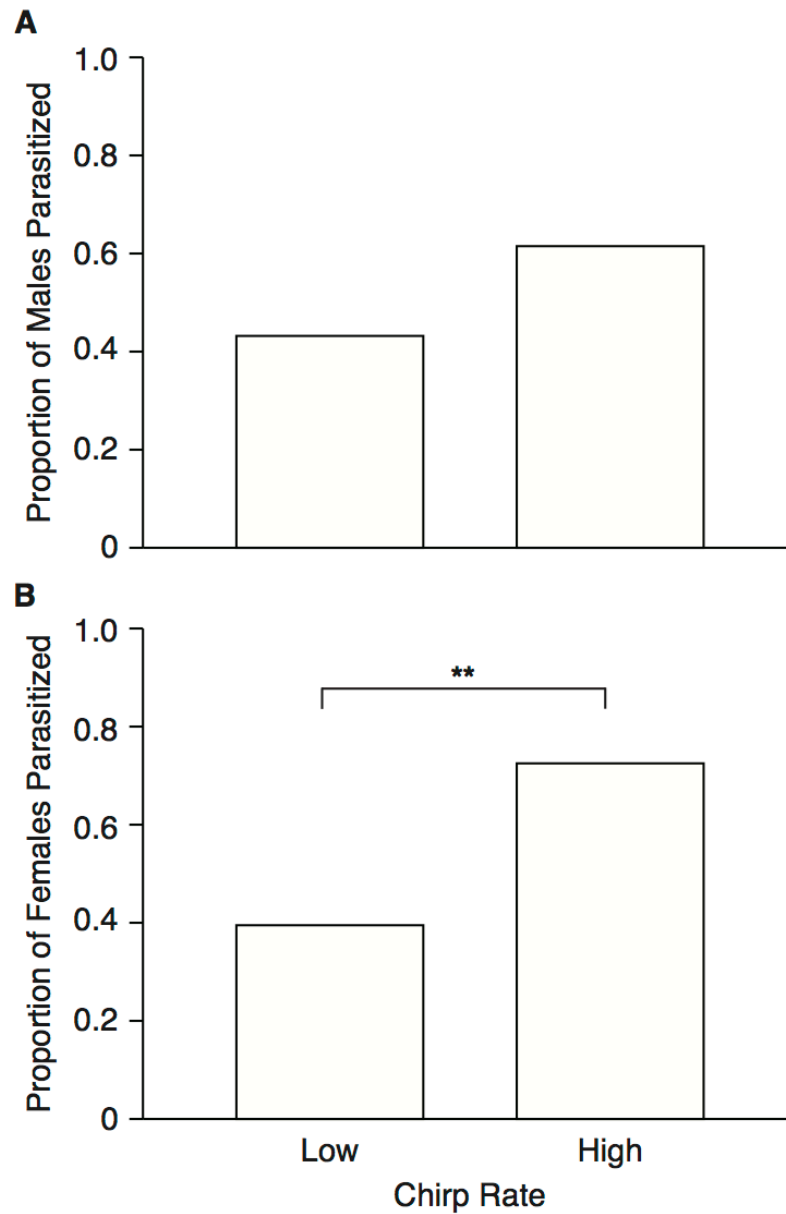
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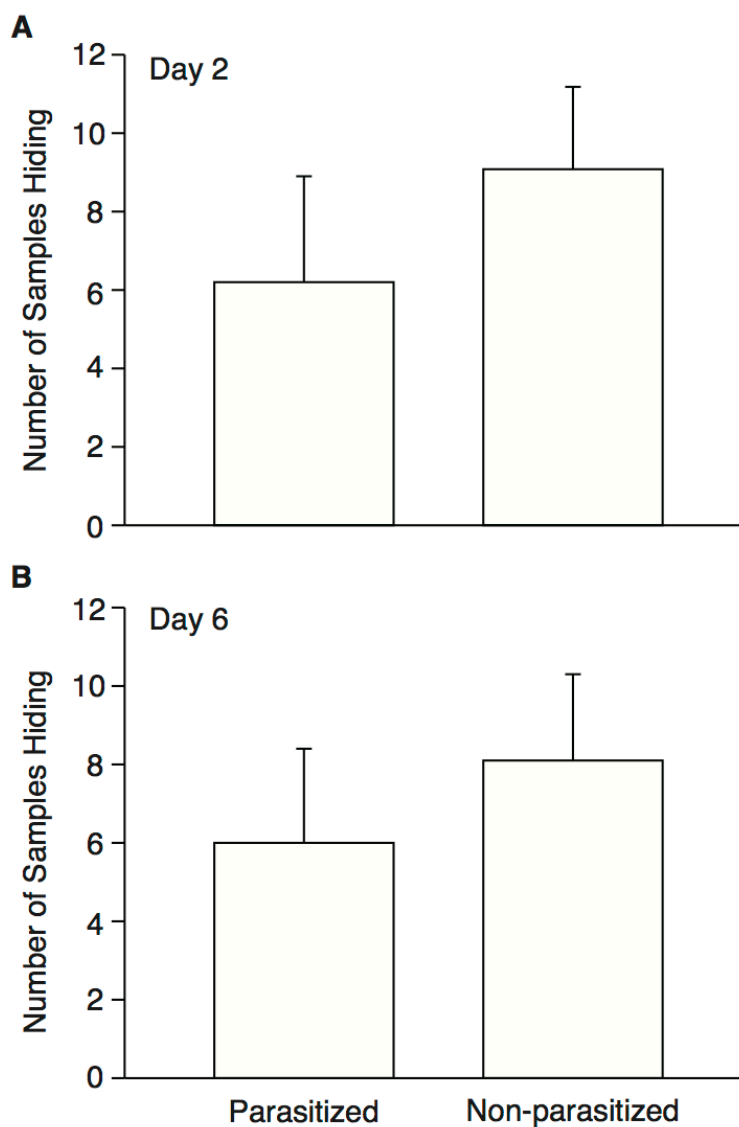
## FIGURES



**Figure 1.1** Proportion of crickets parasitized in the low and high chirp rate treatments.

Male crickets (A) in the high chirp rate treatment tended to be parasitized more than males in the low chirp rate treatment. Female crickets (B) in the high chirp rate treatment were nearly twice as likely to be parasitized than females in the low chirp rate treatment.

Significant differences are indicated by asterisks (\*\*  $p < 0.01$ ).



**Figure 1.2** Number of sampling periods in which female crickets were hiding.

We found no difference in the number of sampling periods in which parasitized and non-parasitized female crickets were hidden under a shelter on either day 2 (A) or day 6 (B) in the activity patterns experiment. Means plus one standard error are shown.

**CHAPTER 2**

**TRAVERSING THE MINEFIELD: FEMALE FIELD CRICKETS THAT  
APPROACH MALES RISK PARASITISM FROM PREVIOUSLY DEPOSITED  
FLY LARVAE**

Cassandra M. Martin

## ABSTRACT

Females may risk predation when near preferred males because the conspicuous signals of these males attract predators. The probability that a predator arrives while a female is near a male should often have the greatest effect on the magnitude of this risk. However, in some systems, a female's risk of predation may extend past the presence of the predator. A parasitoid fly, *Ormia ochracea*, orients to the songs of male field crickets, and shoots larvae on and around the target host. In the variable field cricket, *Gryllus lineaticeps*, previous work has shown that females are parasitized when near males. They may become parasitized when associating with a male if they are present when a fly arrives. They might also risk parasitism if they encounter previously deposited larvae. In this study, we tested the ability of larvae to infect females zero, two, and six hours after being deposited. More than 20% of the test females became parasitized after briefly walking across a substrate with larvae, and there was no difference in the proportion of females infected among the time periods. Because females have a high risk of parasitism from previously deposited larvae, and because this risk remains high long after a fly has deposited larvae around a male, females might be unable to avoid parasitism by shifting their mating activity to a time when the flies are less active, as occurs in some other animals in which predators show diel activity patterns.

## INTRODUCTION

Female animals often prefer males with conspicuous sexual signals (Andersson 1994). These conspicuous signals not only increase the predation risk for males (reviewed in: Burk 1982; Lima & Dill 1990; Sakaluk 1990; Magnhagen 1991; Zuk & Kolluru 1998; Haynes & Yeargan 1999; Robinson & Hall 2002), but can also increase the predation risk for females that associate with them (reviewed in: Lima & Dill 1990; Sakaluk 1990; Jennions & Petrie 1997; Hughes et al. 2012). When preferences carry costs, such as predation risk, there can be large effects on sexual selection. Selection should favor preferences that balance the benefits and costs of choice (Pomiankowski 1987). The costs of choice can override the Fisher model of sexual selection, altering the magnitude and direction of female preference, and in turn, affecting the evolution of mate attraction traits in males (Pomiankowski et al. 1991).

The probability of a female being attacked during mate choice may be low unless females spend long periods of time near signaling males. In some insects, however, eavesdropping parasitoids not only attack signaling males, and females near the males, but also leave larvae in the environment that might infect females that approach males long after the adult parasitoids have departed (Tachinid flies: Cade 1975; Adamo et al. 1995b; Allen et al. 1999; Lehmann 2003). The probability of a female being attacked may thus be substantial, even when the risk is low that a parasitoid will approach when a female is near a male.

The parasitoid fly, *Ormia ochracea*, infects various field cricket species in the family Gryllidae (Cade 1975; Walker 1986; Walker & Wineriter 1991; Zuk et al. 1993; Wagner 1996; Hedrick & Kortet 2006). Male field crickets produce calling songs to

attract conspecific females (Alexander 1960), and the adult female parasitoid flies are attracted to this calling song (Cade 1975). Once the fly has located a singing male, she deposits larvae on and around the male (Cade 1975). Approximately three larvae contact the male, and an average of six larvae may end up on the ground near the male (Adamo et al. 1995b). The larvae that contact a cricket penetrate its cuticle then feed and grow inside of it (Cade 1975; Walker & Wineriter 1991; Zuk et al. 1993; Adamo et al. 1995a). After seven to twelve days, the larvae emerge to pupate, and the host dies (Cade 1975; Walker & Wineriter 1991; Zuk et al. 1993; Martin & Wagner 2014). It is not clear why the flies deposit larvae around males, but one reason could be that male field crickets may attack flies that approach too closely (Martin personal observation). Some tachinid flies, for example, are known to shoot larvae at their hosts from a distance (Allen et al. 1999). The larvae deposited on the ground may thus be a wasteful side effect of the method of deposition. Alternatively, the flies may benefit from depositing larvae on the ground because of collateral infection, including the infection of females attracted to the male, competing males, or passing juveniles (Cade 1975; Walker & Wineriter 1991; Lehmann 2003). Regardless of why larvae are deposited on the ground, selection should favor larval traits that result in high post-deposition survival. If these larvae can survive and remain infective for long periods of time, female crickets attracted to males that have attracted flies may risk parasitism long after a fly has deposited its larvae.

We examined the time over which *O. ochracea* larvae deposited on the ground can infect female variable field crickets, *Gryllus lineaticeps*. Males in this field cricket species produce a chirped calling song to attract females, and females prefer male songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner & Reiser 2000;



Wagner & Basolo 2007b). Male song, however, attracts female *O. ochracea*, which also prefer songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner & Basolo 2007a). Female crickets do not sing and thus do not directly attract flies, but up to six percent of the females present in a population may be parasitized at a given time (Martin & Wagner 2014). In field crickets, males call from the entrance of burrows and females search for them (Alexander 1960). After locating a potential mate, the female will approach to assess him. Female crickets can become directly parasitized when in association with male song above ground, and this risk is higher when near higher chirp rate song (Martin & Wagner 2014). After deciding to mate, the female and male disappear into the male's burrow where the female is unlikely to be directly attacked by a fly. However, females still risk picking up previously deposited larvae on approach and retreat from the male or even from the male himself. *O. ochracea* larvae can live for at least eight hours on moist filter paper (Beckers et al. 2011), and thus could potentially infect females attracted to a male long after they have been deposited, if they remain infective. In this study, we experimentally tested the ability of larvae to successfully infect female crickets immediately following deposition, two hours after deposition, and six hours after deposition.

## **METHODS**

Gravid female parasitoid flies, *Ormia ochracea*, were obtained from Rancho Sierra Vista, part of the Santa Monica Mountains National Recreation Area, near Thousand Oaks, CA, USA. *Gryllus lineaticeps* song was broadcast to attract flies, and the flies were collected using small vials after they landed on the speaker. The flies were then

transported to the laboratory in Lincoln, NE, USA, where they were maintained individually in clear plastic containers (18 x 15 x 11 cm) that contained paper towel strips for substrate and a small cup of food. The food cup was lined with Great Value natural applesauce and contained a piece of cotton wetted with sugar water. The food cups were changed every other day.

The female crickets used in the experiment were third and fourth generation offspring of field-inseminated females from Santa Barbara Shores County Park, Goleta, CA, USA. Matings were managed in the cricket stocks to minimize inbreeding, and full-sibling families were reared in large containers (25 x 15 x 17 cm) that contained a paper towel substrate, two large egg carton shelters, a large cotton-plugged water vial, and ad libitum Purina Cat Chow. At the penultimate molt, females to be used in the experiment were removed from the family containers and placed in smaller individual containers (15 x 8 x 11 cm) that contained a paper towel substrate, one small egg carton shelter, a small cotton-plugged water vial, and ad libitum Purina Cat Chow. Nymphs were held in an environmental chamber maintained at  $23 \pm 1$  °C and 45% RH; once mature, adult females were held in a room maintained at  $23 \pm 1$  °C and 35% RH. Both nymphs and adult females were maintained on a 14:10 light:dark cycle and in acoustic isolation. Females were between 6 and 15 days post maturity when used in the experiment.

Fly larvae were obtained by removing them from adult female flies. The fly was immobilized and sacrificed by piercing its head with a dissection probe. The abdomen of the fly was then removed, and the reproductive tract was excised in one piece. We then stretched out the reproductive tract and cut away the membranes surrounding the planidial larvae to expose them (method adapted from: Vincent & Bertram 2010). We

noted the time the larvae were first exposed as the time when we opened the reproductive tract. We then plated the larvae onto Petri dishes (10 cm diameter) lined with filter paper that had been sprayed five times with tap water from a spray bottle held approximately 30 cm above the dish. Six larvae were placed on each dish by transferring them with a dissecting probe. We used a template in order to place the larvae in the same approximate configuration on all dishes (configuration: one larva each at the points of a larger upright equilateral triangle and at the points of a smaller upside down equilateral triangle situated within the larger one). We established three treatment groups that varied in the time following deposition at which females were exposed to the larvae: zero, two and six hours. We prepared the six-hour dishes first, then the two-hour dishes, and then the zero-hour dishes. After preparation, we checked to make sure all larvae were alive and replaced any that did not appear alive; larvae were considered alive if they waved their anterior end in response to a dissection probe waved in the air above them. The time period officially started after all plates for that time period were prepared and checked, thus the times at which females were exposed to the larvae were a little more than zero, two, and six hours following deposition. The dishes for each time period were placed together in a cardboard box (30 x 22 x 11 cm). The boxes were placed in a dark room maintained at  $20 \pm 2$  °C.

All trials were conducted using red lighting during the cricket's natural mating period. At the beginning of a trial, one of the dishes for the appropriate time period was removed from the box and placed on the work surface; the lid was then removed from the dish. A female cricket was placed in a clear plastic cup with an opening the same circumference as the dish and a piece of cardboard was used to cover the opening. The

cup with cardboard was then inverted on top of the dish, the piece of cardboard was removed, and the trial began when the cricket contacted the larvae-laden filter paper inside the dish. Trials lasted for one minute. In order to entice the female to move around the dish, we tapped the plastic cup with three quick taps on the same side at 15, 30, and 45 seconds. We also broadcast a chorus of five males in the background to simulate mate search conditions and entice the female to move around the dish. Song was broadcast at 68 dB measured 30 cm from the speaker; the speaker broadcasting the male chorus was approximately 45 cm from the experimental dish. After the female had the opportunity to run inside the dish for 60 seconds, we transferred the female to an empty plastic container (25 x 15 x 17 cm) by tipping the dish at a 45-degree angle and allowing the female to run off of the dish into the container. We used this transfer method in order to reduce handling, which might remove larvae attached externally to the female. Thirty minutes later, we transferred the female to her home container, which required handling the female; all attached larvae may have not penetrated the cuticle by this time but waiting thirty minutes reduced our chances of accidentally removing them. The trials were run on three different days; all time periods were represented approximately equally on each day. All the larvae came from one fly each for the first and second day. For the third day, we had to use two flies to get enough larvae to complete the desired number of trials.

Female crickets were monitored for parasitism for 15 days after a trial. Parasitism was indicated by the presence of parasitoid pupae in the cricket's container. Crickets remaining alive with no parasitoid pupae in their container after 15 days were considered not parasitized. We compared the number of females parasitized out of the total number of trials ( $n = 16$  for all time periods) among the three time intervals (zero, two, six hours)

using an exact test. Statistical analyses were performed using STATA Release 11 for Macintosh.

## RESULTS

Female parasitism rates ranged between 19 and 25% for the three time periods, and 21% of all females that traversed the larvae-laden filter papers became parasitized. The likelihood of females becoming parasitized did not differ for larvae deposited zero, two, and six hours prior to the introduction of the female into the test arena (exact test,  $p = 1.00$ ; Fig. 2.1). One larva successfully emerged and pupated from each of the parasitized females.

## DISCUSSION

The risk of predation incurred by female animals when associating with sexually-signaling males might often be low if females only spend a short period of time near signaling males, or if pairs move to safe locations once females have approached males. Some parasitoid flies, however, deposit larvae on the ground around males (Cade 1975; Adamo et al. 1995b). As a result, female crickets might risk parasitism not only when they are near males, but also when traversing a minefield of larvae deposited by a fly long before the female approaches. Female variable field crickets, *Gryllus lineaticeps*, prefer male songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner & Reiser 2000; Wagner & Basolo 2007b), but these song types are also preferred by the parasitoid fly, *Ormia ochracea* (Wagner 1996; Wagner & Basolo 2007a). Previous work has shown that females are parasitized when near singing males (Martin & Wagner

2014), and that fly larvae deposited on the ground can survive for at least eight hours (Beckers et al. 2011). In this study, we tested the ability of larvae deposited on the ground to infect females. We found that *O. ochracea* larvae were capable of infecting female field crickets for up to six hours after deposition. Parasitism rates for our experimental females ranged between 19% and 25%, and there was no significant difference in the likelihood of parasitism for females exposed to larvae deposited zero, two and six hours prior to exposure. These results suggest that females – and perhaps males and juveniles – risk parasitism when they approach males that have been attacked by a fly earlier in a given night.

The long period of survival and infectivity of previously deposited larvae has important implications for the evolution of female cricket mating behavior. Male field crickets begin to sing around dusk and will sing intermittently throughout the night (French & Cade 1987); likewise, female field crickets will search for males throughout the night (French & Cade 1987). The flies, however, are only active for a few hours following sunset (Cade et al. 1996). Because the larvae can live at least eight hours (Beckers et al. 2011) and can infect a cricket for at least six hours (this study), the risk of parasitism for females is substantially longer than the short period during which the flies are active; females can pick up previously deposited larvae throughout most of the night. Females can thus incur parasitism costs when they approach males with preferred song types, even when there is a low probability that a fly will arrive during the short period between when a female approaches a male and when the pair enters the male's burrow. One predicted effect of predation might be a shift in female mating activity to time periods when predators are less active (Moore 2002). In *G. lineaticeps*, females may

benefit little from shifting their mating activity to time periods when flies are not active as the risk from larvae lasts throughout much of the night. It is even possible that the parasitism risk is lowest for females that approach males at sunset, when the flies are just beginning to orient to singing males. This may explain why males in high risk populations sing as frequently in the two hours following sunset as males from low risk populations, despite the cost of doing so (Beckers & Wagner 2012).

It is somewhat surprising that many of the larvae deposited by a fly end up on the ground around a male (Adamo et al. 1995b). This may be an incidental result of the method of larval deposition; because males can kill the flies (Martin personal observation), the flies may larviposit at some distance from males. It is also possible that laying a minefield of larvae is adaptive. Male parasitism rates can be quite high (as high as 60% in some populations, Martin & Wagner 2014), and the number of singing males severely decreases as the fly season progresses (Martin personal observation), thus resulting in substantial competition for non-parasitized, acoustically-located hosts. Also, superparasitism is costly for the flies, but the flies do not seem to be able to detect and avoid already parasitized hosts (Adamo et al. 1995b). Depositing larvae around males may increase parasitism rates by targeting non-parasitized collateral hosts, which should be much more abundant than singing males and perhaps more active than already parasitized crickets. Due to these factors, even a low probability of collateral infection may compensate female flies for the cost of the larvae. *O. ochracea* in Florida carry approximately 200 larvae (Wineriter & Walker 1990), while *O. ochracea* in California carry approximately 300 larvae (Wagner unpublished), which is likely much higher than the number of surviving adults that will be produced from one female fly. Regardless of

whether it is adaptive for female flies to deposit larvae on the ground, selection should favor larval traits that increase the survival and infectivity of larvae that do not immediately contact a host. Selection for these traits should be particularly strong because of the high probability that a larva will experience this condition. It is thus unsurprising that larvae survive and remain infective for long periods of time.

Incidental predation of females is likely to happen in any system in which conspicuous male traits attract predators. This predation risk is usually assumed (but see: Sakaluk & Belwood 1984; Pocklington & Dill 1995; Martin & Wagner 2014); however, the effects of this assumed risk have been investigated in several animals by testing the effect of perceived predation risk on female mating behavior (Forsgren 1992; Endler & Houde 1995; Godin & Briggs 1996; Gong & Gibson 1996; Johnson & Basolo 2003; Velez & Brockmann 2006; Dunn et al. 2008; Bierbach et al. 2011; Bonachea & Ryan 2011). In one of the best-studied systems, male and female guppies in high predation populations show an evolved shift in their mating activities to times when their primary predators are least active (Endler 1987). In the field crickets attacked by parasitoid flies, however, females may be able to do little to escape the latent risk of parasitism other than to preferentially mate with males with less conspicuous traits. While fly parasitism has affected the evolution of male singing behavior in some field crickets (Zuk et al. 2006), it is not yet known whether fly parasitism has affected the evolution of female responses to males.



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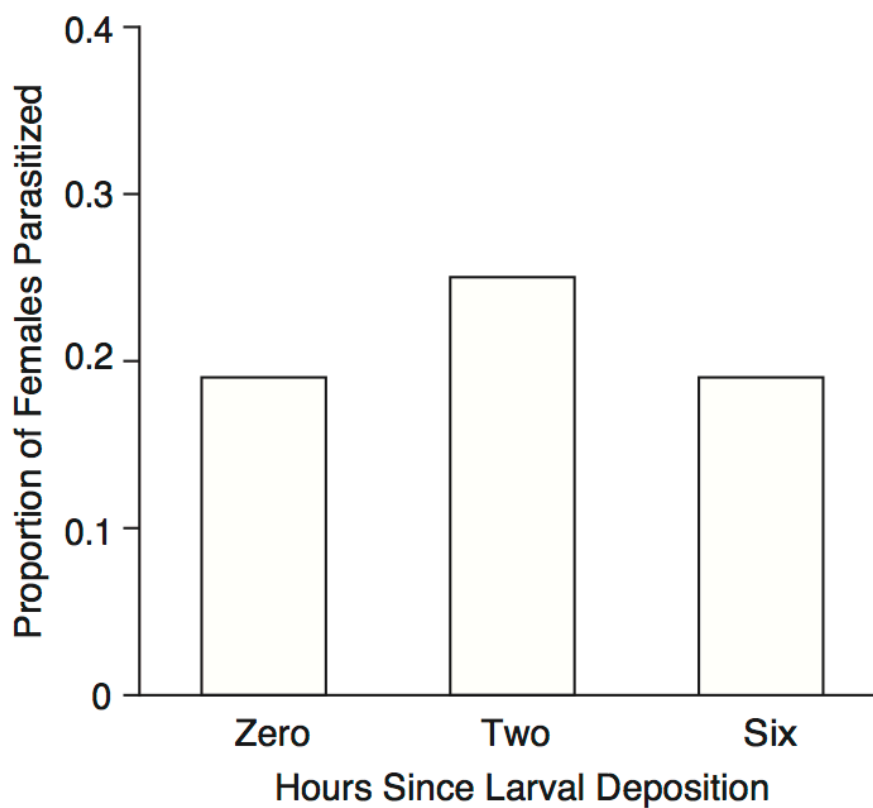
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## FIGURES



**Figure 2.1** Proportion of female crickets parasitized in the different time intervals.

There was no significant difference in the proportion of female crickets parasitized zero, two, and six hours after parasitoid fly larvae were deposited.

**CHAPTER 3**

**INFECTION BY PARASITIDS HAS A GREATER EFFECT ON THE  
FECUNDITIES OF YOUNGER FEMALE FIELD CRICKETS**

Cassandra M. Martin

**ABSTRACT**

Parasitoids have major effects on the reproductive success of their insect hosts. Not only do they eventually kill their hosts, but also, while the hosts are still alive, they use resources that the hosts could potentially use for reproduction. Field crickets are parasitized by the parasitoid fly *Ormia ochracea*. Fly larvae feed on host tissues and kill the cricket upon emergence. We explored the effect of parasitism on the lifetime fecundity of female variable field crickets, *Gryllus lineaticeps*, infected at a younger and an older age. We found that the lifetime fecundity of females infected at a younger age was near zero eggs produced, whereas females infected at an older age produced fewer but some eggs following parasitism. Parasitism is thus particularly costly for young females. Due to these large fitness effects, even low levels of fly predation may favor age-dependent female behaviors that reduce the risk of parasitism.

## INTRODUCTION

Parasites are known to affect the reproductive activities of their hosts (reviewed in: Minchella 1985; Forbes 1993; Forbes 1996). Some animals show a short-term increase in reproduction following parasitism (reviewed in: Agnew et al. 2000). It is thought that these animals increase their reproductive activities following parasitism in order to partially compensate for the reduction in reproduction that occurs as the level of infection increases and as the probability of survival decreases (Forbes 1993; Agnew et al. 2000). More common, though, is for animals to show a decrease in reproduction following parasitism (reviewed in: Baudoin 1975; Hurd 1990b; 1990a). This reduction in reproductive activity could be a byproduct of infection, manipulation of the host by the parasite, or an adaptive strategy of the host to limit further damage (Hurd 2001a).

Parasitoids are insects whose larval stage feeds on a host animal, usually another insect (Godfray 1994). Whereas a single parasite is usually not lethal, a single parasitoid almost always kills its host (Kuris 1974). In addition to a reduced reproductive lifespan, harboring a parasitoid could reduce reproductive activity while the host is still alive. Reduction in reproductive success due to parasitoid infection could have several, non-mutually exclusive causes. Byproducts of parasitoid infection would include tissue damage, reduced nutrient availability, and/or physiological changes (Beckage 1985). Some parasitoids may even directly manipulate the host to stop investing in reproduction, thus freeing up resources for the parasitoid (Libersat et al. 2009). Studies of the effect of parasitoids on host reproduction are less common than those on the effects of parasites, and most of those on parasitoids have been done using parasitoid wasps (reviewed in:



Kraaijeveld & Godfray 2008). Much less is known about the effects of parasitoid flies on host reproduction.

The fly *Ormia ochracea* is a parasitoid of field crickets. The adult female fly orients to male advertisement song and lays larvae on and around its intended host (Cade 1975). Once attached, the larvae burrow inside of the cricket (Cade 1975; Adamo et al. 1995a). For the first three days, the larvae settle in the thoracic muscles, but feed mainly on interstitial fluid (Adamo et al. 1995a). After three days, the larvae move to the abdomen where they feed on the fat body and muscles but largely avoid the reproductive organs (Adamo et al. 1995a). The larvae emerge seven to twelve days later to pupate (Cade 1975; Walker & Wineriter 1991; Martin & Wagner 2014). Upon emergence, they purge their gut contents into the host, and the host dies shortly thereafter (Adamo et al. 1995a).

Because parasitism reduces the reproductive lifespan of the host, it has a negative effect on host fitness. This reduction in host fitness, however, may be magnified if hosts show reduced reproductive activity in the period between parasitism and death. Parasitism is known to affect male reproductive activity. For example, parasitized males spend less time producing calling song (Kolluru et al. 2002; Orozco & Bertram 2004; Beckers & Wagner 2011), and if a female is attracted, they take longer to produce courtship song (Adamo et al. 1995a). These reductions in singing activity likely decrease the probability of attracting a mate and of mating with those females attracted. Other aspects of male reproduction are also negatively affected by parasitism (Kolluru et al. 2002). Male bushcrickets, *Poecilimon marianne*, that were parasitized by the fly

*Therobia leonidei* had a 42% reduction in potential lifetime reproductive success (Lehmann & Lehmann 2006).

In contrast to the known effects of parasitoid flies on the reproductive success of male field crickets, less is known about the effects of parasitoid flies on female reproductive success. Female crickets do not sing, and thus, do not attract flies. However, they do become parasitized (Walker & Wineriter 1991; Adamo et al. 1995b; Martin & Wagner 2014). The primary way in which females are parasitized is likely by walking over previously deposited larvae that were left on the ground near an advertising male (Cade 1975; Adamo et al. 1995b; Martin 2014). Fly larvae deposited on the ground can remain alive for more than eight hours after deposition (Beckers et al. 2011) and can be infectious for at least six hours (Martin 2014). As for males, parasitism may have greater effects on female fitness than just a reduction in reproductive life span. Females in *Gryllus texensis*, *G. rubens*, and *G. bimaculatus* that are infected by fly larvae produce fewer hatchlings than non-infected females, showing a sharp drop in the number of hatchlings three days after being parasitized (Adamo et al. 1995a); this study used females that had been mating and laying eggs for some time before being parasitized. Parasitism almost certainly has larger effects on the fitness of younger females than older females; younger females will lose a larger proportion of their reproductive lifespan if parasitized. Younger and older females might also differ in how reproductive activity changes following parasitism.

In this study, we tested the effects of parasitism by *Ormia ochracea* on the lifetime fecundities of younger and older females of the variable field cricket, *Gryllus lineaticeps*. Female *G. lineaticeps* risk parasitism when near singing males (Martin 2014;

Martin & Wagner 2014). Females in many populations prefer males that produce songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner & Reiser 2000; Wagner & Basolo 2007b), and males with higher chirp rates and longer chirp durations provide seminal fluid products that increase female fecundity and life span (Wagner & Harper 2003). Additionally, the parasitoid fly is attracted to male songs with higher chirp rates and longer chirp durations (Wagner 1996; Wagner & Basolo 2007a; Martin & Wagner 2014). Because of the costs of parasitism, the parasitoid flies may affect the evolution of female preferences. This will be particularly true if parasitism not only reduces female life span, but also reduces female reproduction while still alive. In addition, if younger females have more to lose from becoming parasitized, then the cost of parasitism might favor age-related differences in female song preferences.

To determine how parasitism affects female fecundity and if this differs with age, we infected female *G. lineaticeps* with *O. ochracea* fly larvae at a younger and an older age. Female crickets were paired with males at approximately 10 days of adult age, then separated into four treatment groups: infected younger at approximately 12 days of adult age, sham-infected younger, infected older at approximately 20 days of adult age, and sham-infected older. We compared lifetime fecundity and the temporal pattern of egg laying between younger parasitized females, younger sham-infected females, older parasitized females, and older sham-infected females. We predicted that parasitism would have a larger effect on the fecundity of younger females since older females would have more time to reproduce before becoming parasitized and perhaps before parasitism began to have any potential detrimental effects.

## METHODS

We tested the effect of parasitism and age on female lifetime fecundity in *Gryllus lineaticeps*. The females used were second and third generation offspring of field-caught females from Rancho Sierra Vista, part of Santa Monica Mountains National Recreation Area, near Thousand Oaks, CA, USA. Matings were arranged to minimize inbreeding, and crickets were reared in family groups in clear plastic containers (25 x 15 x 17 cm) that contained a paper towel substrate, two large egg carton shelters, a large water vial plugged with cotton, and ad libitum Purina Cat Chow. At the penultimate molt, both male and female nymphs were transferred to individual clear plastic containers (15 x 8 x 11 cm) with a paper towel substrate, one small egg carton shelter, a small water vial plugged with cotton, and ad libitum Purina Cat Chow. Separation of the sexes prior to maturation ensured that all individuals were virgins before we began the experiment. We recorded the date crickets matured in order to determine adult age (the number of days since the final molt). All crickets were maintained under a reversed 14:10 light:dark cycle. Families were maintained at 22°C, and nymphs were maintained at 30°C. Upon maturity, adult females (24°C) and males (24°C) were housed separately until the experiment commenced. To infect the crickets, we used gravid female flies that were collected from Rancho Sierra Vista and sent back to the laboratory in Lincoln, NE, USA. Flies were housed individually in clear plastic containers (15 x 8 x 11 cm) with shredded paper towel strips and a cup with sugar water-wetted cotton and natural applesauce. The food cups were changed every other day.

Female field crickets have fully formed reproductive organs when they molt into adults and become responsive to male song at approximately 7 days post adult eclosion.

When the females reached  $10 \pm 1$  days of adult age, they were paired with an unrelated male (median male age = 11 days of adult age, range = 9 - 17). In total, 58 females were paired with males. Mated pairs were housed in the smaller individual containers (15 x 8 x 11 cm) with a fresh paper towel, fresh Cat Chow, and the female's egg carton shelter. Each pair was provided with a 15 mL glass vial packed with moist cheesecloth in which to lay eggs (Wagner et al. 2001; Wagner & Harper 2003; Tolle & Wagner 2011). The moist cheesecloth also provided water. The pairs remained together for the duration of the experiment. We conducted two infection treatments (infection and sham-infection) at two female ages:  $12 \pm 1$  days of adult age (2 days following the introduction of the male; younger age group) or  $20 \pm 1$  days of adult age (10 days following the introduction of the male; older age group). In the younger age group, 14 females were infected and 14 females were sham-infected. In the older age group, 15 females were infected and 15 females were sham-infected.

To obtain fly larvae, adult female flies were sacrificed by piercing their head with a sharp dissection probe, the abdomen was then removed, and larvae were dissected from the reproductive tract (for specific details, see Vincent & Bertram 2009). For the infected groups, we placed larvae on the soft tissue between the pronotum and wings using a dissection probe. In order to expose this soft tissue, we lightly pinched the female between the fingers of one hand and more roughly tilted the pronotum and head forward with the other hand. The goal was to successfully transfer two larvae to each female. For the sham-infected groups, we handled the females as we did the infected females, and then lightly rubbed their soft tissue with a clean dissection probe four times (it often took more than two attempts to transfer two larvae, so 4 rubbings was more representative of

the larval transfer procedure). Females were monitored for the emergence of parasitoid pupae to determine if infection was successful. Pupae did emerge from all infected females (median = 1 pupa, maximum = 3).

Egg vials were collected every other day beginning after the female was paired with the male. We counted the number of eggs laid in each vial. The total number of eggs laid for 20 days after being paired with a male (or until death for infected females) was our measure of lifetime fecundity. Females were about 30 days post-maturity using this measure; this approximates the maximum natural life span of female field crickets (Murray & Cade 1995). Infected females in the younger age group lived and had egg vials collected for up to 14 days after being paired with a male; infected females in the older age group lived and had egg vials collected for up to 20 days after being paired with a male. Again, both control groups had egg vials collected for 20 days after being paired with a male.

We initially collected eggs for 26 days (about 36 days post-maturity), but subsequently decided to only include eggs produced within a reasonable approximation of the maximum natural life span. Some females, however, laid eggs outside of the vials, and some eggs dried up and fell out of their original vials. Because a female's egg vials were stored as a group before the eggs were counted, the loose eggs could not be assigned to a particular day and could have been laid after our lifetime fecundity cut-off for sham-infected females. Thus, loose eggs were excluded from the lifetime fecundity counts for sham-infected females. Loose eggs were included for infected females because they all died, and thus laid eggs, before the lifetime fecundity cut-off. There were four females who had loose eggs that comprised less than 10% of their totals (median = 2%

loose eggs, maximum = 7%) and were included in the dataset; two females with 10% or greater loose eggs were discarded from the dataset. Loose nymphs, in contrast, almost certainly came from earlier vials because later eggs could not have hatched by the time we counted the eggs; thus, loose nymphs were included in our lifetime fecundity totals for all females included in the dataset. Our final sample size for lifetime fecundity was 14 infected and 14 sham-infected females in the younger age group and 15 infected and 13 sham-infected females in the older age group. For examining 'daily' fecundity patterns, loose eggs and nymphs were unable to be assigned to a particular day. These unassigned offspring were not included in the 'daily' counts. Eight females with unassigned offspring comprising less than 10% of her total were included in the examination of daily fecundity (median = 2% unassigned offspring, maximum = 7%); six females with 10% or greater unassigned offspring were discarded from the analysis. Our final sample size for 'daily' fecundity was 13 infected and 13 sham-infected females in the younger age group and 14 infected and 12 sham-infected females in the older age group.

The lifetime fecundity measures had a highly non-normal distribution. Thus, we used negative binomial regression to test the effects of parasitism, age and the interaction of parasitism and age on female lifetime fecundity. Statistical analyses were performed using STATA Release 11 for Macintosh. Daily fecundity patterns were only visually compared.

## RESULTS

Female lifetime fecundity was significantly affected by parasitism status ( $z = 3.15, p = 0.002$ ) and age group ( $z = 2.98, p = 0.003$ ). Sham-infected females produced

more eggs than infected females, and females that had their parasitism status manipulated at an older age produced more eggs than females that had their parasitism status manipulated at a younger age. The interaction between parasitism status and age group was not included in the final model because it was non-significant. One infected female in the younger age group laid 100 eggs, whereas all other infected females in the younger age group laid 9 or fewer eggs. Because this female had the potential to substantially bias the results, we excluded this female and re-analyzed the data. As with the initial analysis, female lifetime fecundity was significantly affected by parasitism status ( $z = 5.41$ ,  $p < 0.001$ ) and age group ( $z = 5.35$ ,  $p < 0.001$ ). Sham-infected females produced more eggs than infected females, and females that had their parasitism status manipulated at an older age produced more eggs than females that had their parasitism status manipulated at a younger age (Fig. 3.1). In addition, there was a significant effect of the interaction between parasitism status and age group ( $z = -2.55$ ,  $p = 0.011$ ). The proportional decrease in lifetime fecundity was greater for females infected at a younger age: the median decrease in lifetime fecundity for younger infected females (relative to younger sham-infected females) was 100% while the median decrease in lifetime fecundity for older infected females (relative to older sham-infected females) was 93%.

The temporal pattern of egg laying for females in each group is shown in Figure 3.2. For females in the younger age group, the median number of eggs laid every two days was near zero for both sham-infected (Fig. 3.2 A) and infected (Fig. 3.2 B) females prior to the manipulation on day 2. After manipulation, the females in the sham-infected group showed a linear increase in egg laying beginning 10 days post-manipulation through the remaining 8 days of egg collection (Fig. 3.2 A), while the females who were



infected with parasitoid larvae laid almost no eggs in each two-day period until death (Fig. 3.2 B). For females in the older age group, the median number of eggs laid every two days increased linearly for both sham-infected (Fig. 3.2 C) and infected (Fig. 3.2 D) females prior to the manipulation on day 10. After manipulation, the females in the sham-infected group showed an increase in egg laying for 4 days post-manipulation followed by a decrease for the remaining 6 days of collection (Fig. 3.2 C), while the females who were infected with parasitoid larvae laid almost no eggs in each two-day period until death (Fig. 3.2 D).

## DISCUSSION

Female *G. lineaticeps* infected with parasitoid larvae had reduced lifetime fecundity, and the reduction in fecundity was larger for females infected at a younger age than for females infected at an older age. Younger females thus appear to incur severe costs of parasitism; not only do they have a substantially shorter reproductive life span than non-parasitized females, they also produce extremely few eggs once parasitized.

The age at which females were manipulated had a major effect on lifetime fecundity; females manipulated at an older age produced more eggs than females manipulated at a younger age. The fact that females sham-infected when younger produced fewer eggs than females sham-infected when older is paradoxical; sham-infected females in the two age groups should have produced approximately the same number of eggs. This result suggests that the manipulation (restraining and lightly poking the sham-infected females at the site of infection) had a negative effect on lifetime fecundity. This is further evident in the temporal pattern of egg laying in relation to the

time of sham-infection (Fig. 3.2 A&C). After sham-infection, females in the younger age group (most of which had not yet started producing eggs) produced very few eggs for ten days following the manipulation; then, their egg laying increased in a way that resembled the pattern in older sham-infected females prior to manipulation (days 6 to 10 in Fig. 3.2 C). Furthermore, females sham-infected when older did not maintain the positive linear increase in egg laying that they had prior to manipulation; this reduction, per say, could have been due to naturally approaching peak fecundity (due to aging) or the manipulation. Because the manipulation procedure appears to have affected female egg laying, an accurate assessment of the lifetime fecundity costs of parasitism for younger and older females is not possible. That is, while parasitized females clearly incurred a fecundity cost, the magnitude of this cost cannot be determined from our experiment. For example, non-parasitized females that are not manipulated (a negative control) might produce substantially more eggs than non-parasitized females that are manipulated (the positive control used in our experiment). Thus, the magnitude of the cost could be larger than that demonstrated here. It is also important to note that naturally parasitized females might produce more eggs following parasitism than our manually parasitized females if our manipulation was more intrusive than natural parasitism.

For both our younger and older age groups, the number of eggs laid dropped to almost zero eggs immediately after infection. Female *G. lineaticeps*, like females in other species of field crickets, thus do not appear to compensate for the reduced future reproductive success that results from parasitism by increasing their current reproductive effort (see Vincent & Bertram 2010 for discussion of the reproductive compensation hypothesis in this system). The immediate reduction in egg laying, however, contrasts

with results from related cricket species hand-infected by *O. ochracea* larvae, which laid eggs at levels similar to control females for at least a few days after infection (Adamo et al. 1995a; Adamo 1999). Interestingly, similar differences are seen in males of the different species. In many species of field crickets, males continue to sing normally for a number of days after they are parasitized (Cade & Wyatt 1984; Kolluru 1999; Orozco & Bertram 2004). In *G. lineaticeps*, however, male singing activity drops substantially immediately following parasitism (Beckers & Wagner 2011). These differences between species in the effects of parasitism may result from differences in the biology of the field crickets, differences in biology of the parasitoids in different geographic regions, or both.

The reason that parasitism affects female fecundity is not clear (Adamo et al. 1995a; Adamo 1999). The larvae avoid feeding on reproductive tissues and do little damage to them, so direct damage to the ovaries is not responsible (Adamo et al. 1995a). Adamo et al. (1995a) suggest that nutritional depletion or endocrine manipulation of the host by the parasitoid could potentially account for the fecundity reduction. The large effect of parasitism on female fecundity (this study) and male singing activity (Beckers & Wagner 2011) immediately following infection, however, suggests that nutritional depletion might be an insufficient explanation; it seems unlikely that the small larvae could use enough of the host's nutritional reserves in the first few days following infection to prevent female egg laying and substantially reduce male singing. Endocrine disruption may be more important in our system. In reviewing two well-studied parasite/insect host systems, Hurd (2001b) concluded that interference with the host endocrine system, and not nutrient competition, was likely to account for reduced host reproductive success due to parasitism.

The survival cost of parasitism may be negligible if non-parasitized females also have a low probability of survival in the field. However, in addition to the survival cost, there is a major reproductive cost while females are still alive. Thus, even a relatively low rate of female parasitism may have a significant impact on the evolution of female mating behavior in parasitized populations. Our previous work has shown that females risk parasitism when they approach singing males, and that they have a higher risk near males with preferred song types (Martin & Wagner 2014). Due to the large reproductive cost we found in this current study, females in our species should avoid approaching and mating with males with more risky song types. Additionally, since costs were higher for younger females, there might be age-related variation in risk-sensitivity with younger females exhibiting weaker preferences and older females exhibiting stronger preferences. Avoidance of usually preferred song types could, over time, result in population level shifts in female preference; thus, parasitism of female field crickets may have a strong influence on the evolution of female preferences and, in turn, male sexually selected traits.

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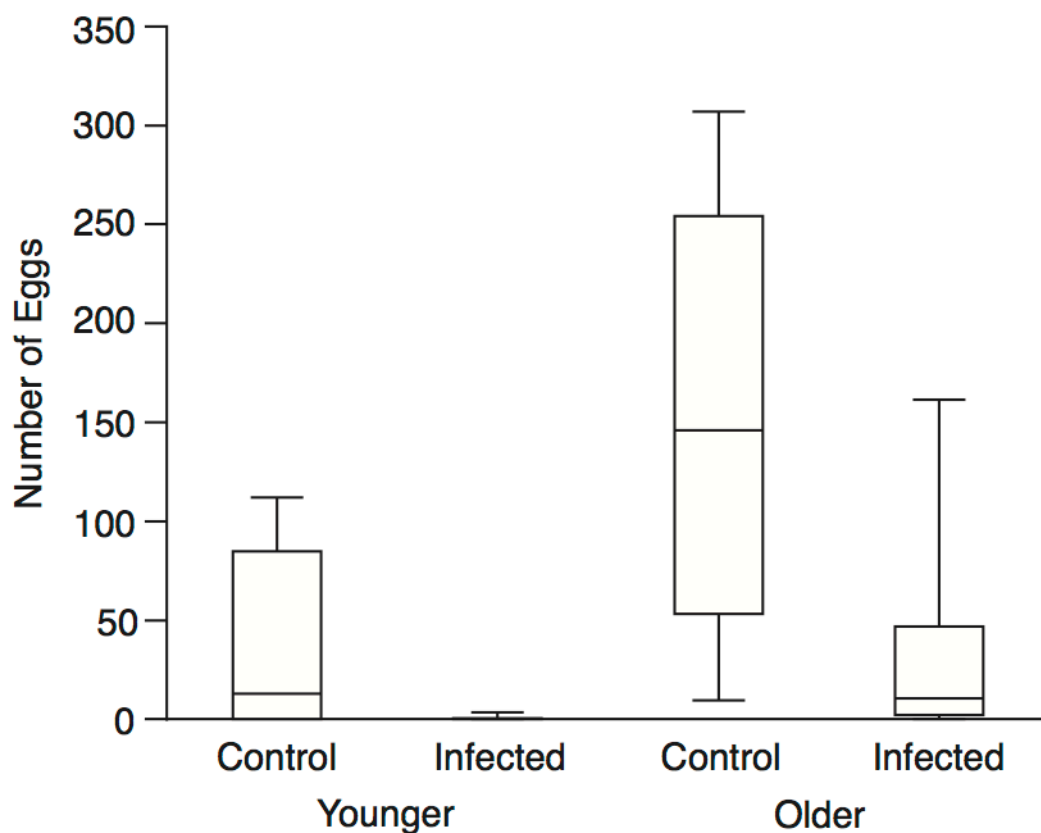
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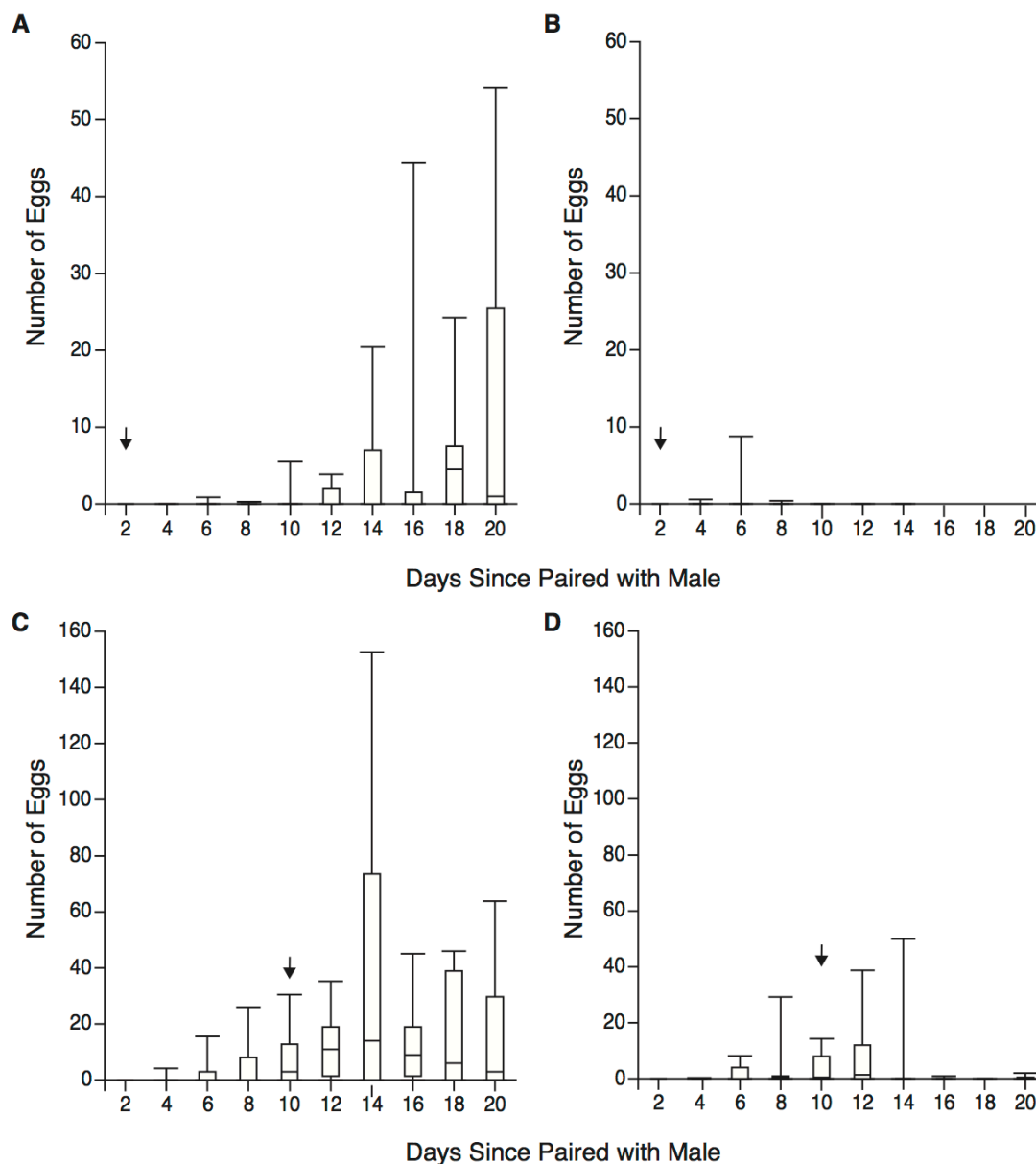
## FIGURES



**Figure 3.1** Lifetime fecundity for female crickets in the experiment.

Box plots of the number of eggs laid by female crickets over the course of the experiment (= lifetime fecundity) are shown. The top line of the box indicates the 75 percentile, the middle line the 50 percentile (median), and the bottom line the 25 percentile; the whiskers indicate the 90 percentile (above box) and 10 percentile (below box). Females were either sham-infected (control) or infected with parasitoid larvae when younger ( $12 \pm 1$  days post adult age) or older ( $20 \pm 1$  days post adult age).





**Figure 3.2** Daily fecundity for female crickets in the experiment.

Box plots of the number of eggs laid every two days by female crickets are shown. The top line of the box indicates the 75 percentile, the middle line the 50 percentile (median), and the bottom line the 25 percentile; the whiskers indicate the 90 percentile (above box) and 10 percentile (below box). Eggs vials were collected every two days after each female was paired with a male; the arrow indicates the day of treatment. Females were either sham-infected when younger (A), infected when younger (B), sham-infected when older (C), or infected when older (D). Note: the vertical axis is not the same scale the for younger and older graphs.

**CHAPTER 4****FEMALES IN A FIELD CRICKET BALANCE THE DIRECT BENEFITS OF  
EXPRESSING PREFERENCES AND THE RISK OF DEATH FROM  
PARASITOIDS**

Cassandra M. Martin

**ABSTRACT**

Females often prefer to mate with males with conspicuous signals because they directly or indirectly benefit. Conspicuous signals, however, often attract predators, and females may experience a higher risk of predation when assessing and mating with preferred males. These opposing sources of selection may result in signal preferences that balance the benefits and costs of mating with conspicuous males. In the variable field cricket, *Gryllus lineaticeps*, females receive direct benefits from males that produce higher chirp rate song, but in some populations, these males are more likely to attract parasitoid flies. Because females can be parasitized when they approach males that attract parasitoids, mating with males that produce high chirp rate song can be risky for females. We investigated female chirp rate preferences in a parasitized population using simultaneous choice tests. We tested the hypothesis that female preferences reflect a balance between the benefits and costs of mating with high chirp rate males. We found that females discriminated against low chirp rate song, but females did not discriminate between intermediate and high chirp rate songs. These results suggest that fly predation may affect the evolution of female preferences in field crickets.

## INTRODUCTION

Female mate choice often represents a balance between the benefits and costs of being choosy. Female animals choose males with certain traits because these males provide the female with indirect or direct benefits (Andersson 1994). However, females may incur costs because of their preferences. When predators and parasitoids eavesdrop on male advertisement signals (reviewed in: Burk 1982; Lima & Dill 1990; Sakaluk 1990; Magnhagen 1991; Zuk & Kolluru 1998; Haynes & Yeargan 1999; Robinson & Hall 2002), the females that respond to the advertisement signals may also be at risk (reviewed in: Lima & Dill 1990; Sakaluk 1990; Jennions & Petrie 1997; Hughes et al. 2012). In some cases, males that produce signals that are most attractive to females are more likely to attract predators and parasitoids. As a result, females may be at greater risk when they approach males with more preferred signals than males with less preferred signals, and this could affect the evolution of female preferences. Current evidence suggests that females from many animal groups avoid conspicuous, usually preferred males when perceived predation risk is high (Forsgren 1992; Godin & Briggs 1996; Gong & Gibson 1996; Gong 1997; Johnson & Basolo 2003; Bonachea & Ryan 2011); however, less is known about whether female preferences have been affected by predation on an evolutionary scale.

Costs incurred while near potential mates are known as association costs. One group of animals in which females appear to incur association costs is field crickets, which are attacked by parasitoid flies. Male field crickets sing to attract mates (Alexander 1961). The song of some male field crickets also attracts lethal parasitoid flies, *Ormia ochracea* (Cade 1975). The flies lay larvae on and around the males (Cade 1975; Adamo

et al. 1995b); these larvae burrow into the cricket where they feed and grow (Adamo et al. 1995a). The cricket dies shortly after larval emergence, which occurs seven to twelve days post infection (Wineriter & Walker 1990; Martin & Wagner 2014). Females are silent, and thus should not attract flies, but they do become parasitized (Walker & Wineriter 1991; Zuk et al. 1993; Adamo et al. 1995b; Martin & Wagner 2014). The most likely mode of parasitism of female crickets is by previously deposited larvae around a singing male. The flies leave an average of six larvae around their intended host (Adamo et al. 1995b), and these larvae can live for more than eight hours (Beckers et al. 2011) and remain infectious for at least six hours (Martin 2014). Females are vulnerable to fly predation by picking up these larvae while assessing and mating with males previously visited by flies. Fly predation could thus affect the evolution of mating behavior in female field crickets. Indeed, female *Gryllus rubens* field crickets were much less responsive to male song in the fall, when flies were active, than in the spring, when flies were not active (Velez & Brockmann 2006).

In the variable field cricket, *Gryllus lineaticeps*, females in at least some populations prefer male song with higher chirp rates (Wagner 1996; Wagner & Reiser 2000; Wagner & Basolo 2007b). Males with high chirp rate song provide females with beneficial seminal fluid products; nutritionally stressed females that are mated to high chirp rate males lay more eggs than those mated to low chirp rate males (Wagner et al. 2001; Wagner & Harper 2003). *O. ochracea* also prefers male song with higher chirp rates (Wagner 1996; Wagner & Basolo 2007a) and females in association with high chirp rate song are nearly twice as likely to become parasitized as females in association with low chirp rate song (Martin & Wagner 2014). Given the costs of parasitism, *G.*

*lineaticeps* females may avoid high chirp rate males in order to reduce their risk of parasitism. Instead, selection might favor females that prefer males with intermediate chirp rate songs as a compromise between mate quality and parasitism risk, or prefer the less risky males with low chirp rate songs.

We examined mate choice in female *Gryllus lineaticeps* from a heavily parasitized population. In a survey of this population, nearly sixty percent of males were parasitized (Martin & Wagner 2014). Thus, females incur a relatively high risk of parasitism when they approach males for mating, particularly when they approach high chirp rate males (Martin & Wagner 2014). We used two stimulus choice tests to examine female chirp rate preferences. A previous study using single stimulus presentations suggested that females strongly respond to intermediate and high chirp rates, but only weakly respond to very high chirp rates (Wagner & Basolo 2007b). Single stimulus presentations test whether females are willing to bypass a male producing a given song type in favor of continued searching. These decisions can be affected by the costs and benefits of mating with a male with a given song type (e.g., predation-related association costs), but also the costs and benefits of searching for additional males. For example, if there are high search costs, females might be more likely to settle for a lower benefit male. In contrast, two speaker choice tests indicate whether, given two alternatives and no differential search costs, females prefer one male to the other.

## **METHODS**

We measured female choice by presenting female crickets with a choice between a pair of song stimuli varying only in chirp rate. We used third and fourth generation

offspring bred from field-inseminated female crickets collected from Rancho Sierra Vista, part of Santa Monica Mountains National Recreation Area, near Thousand Oaks, CA, USA. Matings were arranged to minimize inbreeding, and crickets were reared in family groups in clear plastic containers (25 x 15 x 17 cm) that contained two large egg carton shelters, a paper towel substrate, a large water vial plugged with cotton, and ad libitum Purina Cat Chow. At the penultimate molt, nymphs were transferred to individual clear plastic containers (15 x 8 x 11 cm) with one small egg carton shelter, a paper towel substrate, a small water vial plugged with cotton, and ad libitum Purina Cat Chow. Juvenile crickets were reared in acoustic isolation in an environmental chamber maintained at 32° C. Upon maturity, adult females were moved to an acoustic isolation room maintained at 23° C. All crickets were maintained on a reversed 14:10 h light:dark cycle. Since females were removed from their families before maturity and housed in acoustic isolation thereafter, they were acoustically-naïve virgins.

We tested female preferences in a 2.2 X 2.2 X 2.7 m chamber lined with foam to minimize echoes. The chamber was illuminated with red lighting (four bars of fluorescent lights covered with red film) to facilitate observations but not disturb the crickets. On opposite corners of the floor of the chamber, there were 0.26 m diameter circles denoting the zone where the female was said to be associating with the song. The speakers broadcasting the male songs were set in the center of these circles and placed so that they were 0.31 m away from the wall of the chamber. We broadcasted synthetic songs from a Macintosh Quadra 840 AV using SoundEdit 16. The computer was attached to Optimus SA-155 amplifiers connected to KLH 970 speakers. We broadcast song at 76 dB SPL (re:

20  $\mu\text{Pa}$ ) at 30 cm from the speaker measured using a Radio Shack 33-4050 analog sound level meter. Chamber temperature was maintained at 23° C.

We created synthetic cricket songs by digitizing a natural cricket pulse, copying the chirp eight times to create one 120 ms long chirp (pulse duration = 11 ms, dominant frequency = 5.17 kHz), and then using the chirp to create songs of various chirp rates (see Wagner & Reiser 2000 for details). We used three songs that varied only in chirp rate: a low chirp rate song (1.8 chirps/s), an intermediate chirp rate song (3.0 chirps/s), and a high chirp rate song (4.2 chirps/s). Each test consisted of presenting two of the three songs to a female. Within a given pair of songs, the side from which each song was broadcast was alternated among trials to eliminate any potential side biases. The three song pairs used were: low vs. intermediate chirp rate, low vs. high chirp rate, and intermediate vs. high chirp rate.

To begin a trial, a female was placed under an opaque cup in the center of the arena, equidistant from the two speakers. The appropriate songs for the selected test comparison were then broadcast from the two speakers for 10 minutes while the female acclimated to test conditions. After 10 minutes of acclimation, we picked up the cup and released the female. We then tracked her movements in the arena by observing her on a Panasonic CT-1384Y television outside of the chamber connected to a Panasonic WV-BP100 video camera located on the ceiling of the chamber. Females were given 10 minutes to move around the arena after they first moved from the starting point in the center. During these 10 minutes, we recorded which circle the female first entered and the total time spent in each circle.



Within each pair of songs, we compared counts of which stimulus the female approached first using a binomial test. Females that did not enter a circle were excluded from this analysis; thus, the resulting samples for the choice tests were 28 females for low vs. intermediate chirp rate song, 27 for low vs. high chirp rate song, and 28 for intermediate vs. high chirp rate song. Within each pair of songs, we also compared the time females spent in the circles surrounding each speaker using Wilcoxon signed-rank tests. Only females who spent a combined total of 5 sec in the two circles were used in the time analyses. Not all females that chose a song met the time criteria, thus the resulting sample sizes for the time analyses were 28 females for low vs. intermediate chirp rate song, 26 for low vs. high chirp rate song, and 26 for intermediate vs. high chirp rate song. Statistical analyses were performed using STATA Release 11 for Macintosh.

## RESULTS

Females discriminated against the low chirp rate song, but did not discriminate between the intermediate and high chirp rate song. First, females were more likely to first approach the intermediate chirp rate song than the low chirp rate song ( $p < 0.001$ , Table I), and there was a non-significant trend for females to first approach the high chirp rate song than the low chirp rate song ( $p = 0.061$ , Table 4.1). There was no difference, however, in the number of females that first approached the high chirp rate song and the intermediate chirp rate song ( $p = 0.286$ , Table 4.1).

Second, females spent more time near the intermediate chirp rate song than the low chirp rate song ( $z = -4.35$ ,  $p < 0.001$ , Fig. 4.1 A), and more time near the high chirp rate song than the low chirp rate song ( $z = -2.17$ ,  $p = 0.030$ , Fig. 4.1 B). Females did not

differ, however, in the time they spent near the high chirp rate song and intermediate chirp rate song ( $z = -0.10$ ,  $p = 0.919$ , Fig. 4.1 C).

## DISCUSSION

Female animals can incur predation costs when near males with conspicuous sexual displays; these association costs should favor the evolution of weaker female preferences. In the variable field cricket, *Gryllus lineaticeps*, females tend to prefer higher chirp rate songs (Wagner 1996; Wagner & Reiser 2000; Wagner & Basolo 2007b). We tested the hypothesis that females from a population heavily parasitized by a parasitoid fly may have weaker chirp rate preferences. Our results suggest that females strongly discriminate against low chirp rate songs, but do not discriminate between intermediate and high chirp rate songs. First, females were more likely to approach, and spent more time near, intermediate and high chirp rate song than low chirp rate song. Second, females were not more likely to approach, and did not spend more time near, high chirp rate song than intermediate chirp rate song.

The lack of discrimination between intermediate and high chirp rate song may suggest that parasitism has favored weaker female preferences. However, to determine whether females from parasitized populations are less discriminating than females from non-parasitized populations, we would need to show that females from non-parasitized populations prefer high to intermediate chirp rate song. Some such studies have been done, but they may not make the best comparison. Females from a population with a lower risk of parasitism were tested in a study with a similar two-stimulus choice design; the females preferred the higher chirp rate song in all song combinations they were

presented, including discriminating between the intermediate and high chirp rate songs used in this study (Wagner & Reiser 2000). Results from another study on this population using a single stimulus design indicated that females preferred higher than average chirp rates, but did not prefer very high chirp rates (Wagner & Basolo 2007b). This population, however, has had a variable history of predation risk; it was parasitized during a period after the habitat had been burnt by fire, but the flies disappeared after the habitat recovered (Wagner personal observation). Females from a population that has been known to be at low risk of parasitism for over 15 years (Wagner personal observation), actually showed similar preferences to the females from this study (discriminating against low but not between intermediate and high chirp rates); however, the methods were not directly comparable as females were given a choice between three chirp rate stimuli in each test (Beckers & Wagner 2011). For direct comparison, we would need studies using a similar two-stimulus choice design to this study. Our study indicates that females from one very high risk population do not discriminate between intermediate and high chirp rates, which is consistent with what we would expect if parasitism affects female preferences; however, a comparative study using several populations of known parasitism risk would be the best way to demonstrate whether parasitism affects the evolution of the strength and direction of female preferences.

As survival costs of male sexual signals can limit male traits from becoming too extreme, survival costs of female mating behavior can limit female preferences for these male traits. In several species, predation risk has been shown to reduce current female preferences (Forsgren 1992; Gibson & Bachman 1992; Godin & Briggs 1996; Gong & Gibson 1996; Gong 1997; Johnson & Basolo 2003; Velez & Brockmann 2006; Bonachea

& Ryan 2011); however, the logical first step of quantifying the risk to females when they associate with conspicuous males has not been taken. In *G. lineaticeps*, we know that 1) females can become parasitized when near singing males (Martin 2014; Martin & Wagner 2014), 2) the flies prefer higher chirp rate songs (Wagner 1996; Wagner & Basolo 2007a; Martin & Wagner 2014), and 3) females near preferred song incur increased parasitism risk (Martin & Wagner 2014). The results presented here and elsewhere (see Wagner & Basolo 2007b) suggest that association costs may have affected the evolution of female mating preferences in a field cricket.

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## TABLES

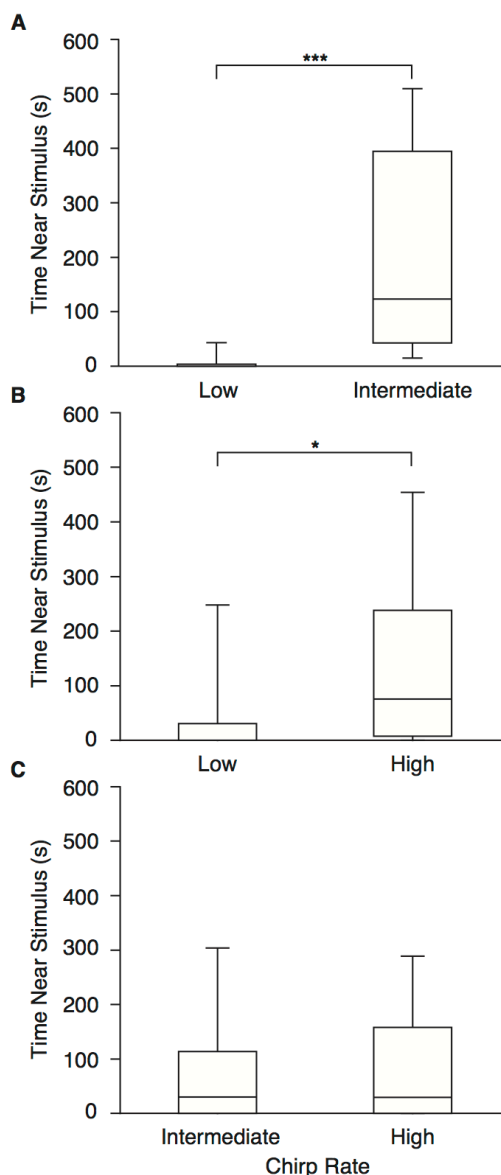
**Table 4.1** The number of female crickets choosing each song type in each pair.

The number of female crickets first choosing the lower or higher chirp rate song when presented with a choice between a pair of songs differing only in chirp rate. The first song type chosen was significantly more likely to be the higher chirp rate song when presented with low and intermediate chirp rate songs ( $p < 0.001$ ). There was a non-significant trend for the first song type chosen to be the higher chirp rate song when presented with low and high chirp rate songs ( $p = 0.061$ ). There was no difference in first song type chosen when presented with intermediate and high chirp rate song.

Test Comparison	First Choice Lower	First Choice Higher
Low vs. Intermediate	4	24
Low vs. High	9	18
Intermediate vs. High	16	12



## FIGURES



**Figure 4.1** The time female crickets spent near each song type in each pair.

Box plots showing the time that female crickets spent with pairs of songs that differed only in chirp rate. The top line of the box indicates the 75 percentile, the middle line the 50 percentile (median), and the bottom line the 25 percentile. The whiskers indicate the 90 percentile (above box) and 10 percentile (below box). Females were presented with low versus intermediate chirp rate song (A), low versus high chirp rate song (B), and intermediate versus high chirp rate song (C). Significant differences are indicated by asterisks (\*  $p < 0.05$ , \*\*\*  $p < 0.001$ ).