IOWA STATE UNIVERSITY
Digital Repository

2014

How male attractiveness mediates the effect of an immune challenge on reproductive traits and sickness behavior in the Texas Field Cricket (Gryllus texensis)

Melissa Shari Corona Telemeco
Iowa State University

**How male attractiveness mediates the effect of an immune
challenge on reproductive traits and sickness behavior
in the Texas Field Cricket (*Gryllus texensis*)**

by

Melissa S C Telemeco

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:
Clint D Kelly, Co-Major Professor
Amy L Toth, Co-Major Professor
Lyric C Bartholomay

Iowa State University

Ames, Iowa

2014

DEDICATION

To my loving husband, Dr. Rory S Telemeco. For your unending patience, advice, and encouragement, I dedicate this work to you. Your kindness, creativity, and perseverance inspire me to be a better person. This thesis would not exist without your support. Thank you.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

ABSTRACT

Trade offs between life-history traits are well studied; however, most studies examine only two life-history traits at a time. For instance, in invertebrates, there is much evidence for the trade-off between reproduction and immunity but no studies that consider how investment in attractiveness might mediate this trade-off. In this study, I test two hypotheses. First, attractive male crickets (*Gryllus texensis*) have acquired larger resource pools than unattractive males and can invest more in all life-history traits. Attractive males should therefore have better body condition and show a less pronounced trade-off than unattractive males. Second, that attractive and unattractive males start with similar sized resource pools and allocate these resources differently. Since attractive males have invested some of their limited resources on sexual attractiveness they should have fewer resources left at their disposal than the unattractive males and should have worse body condition and show a more pronounced trade-off between reproduction and immunity than unattractive males. I tested these hypotheses by comparing body condition and testes size in attractive and unattractive males and injecting them with saline or LPS and examining their behavior. Attractive and unattractive males did not differ in their body condition or testes size. Attractive males increased their calling activity when immune-challenged. These results support the hypothesis that attractive and unattractive males have similar sized resource pools and attractive males invest in sexual attractiveness at the expense of immune-competence.

CHAPTER 1

INTRODUCTION

A central tenet of life history theory is that organisms must divide their limited resources among the competing demands of fitness-related traits such as maintenance, growth, reproduction, longevity, and immunity (Stearns 1992, Roff 1993, Reznick et al 2000). This consequently results in trade-offs within individuals among their life history traits such that increased investment in reproduction, for example, will inevitably result in fewer available resources for other traits such as immunity (Jones 1990, Stearns 1992, Reznick et al 2000, Lawniczak 2007).

Predicting how fitness-related traits should relate to immune function becomes more complicated when we consider inter-individual differences. van Noordwijk and de Jong's model (1986) shows that the correlation between two life history traits might be positive or negative depending on how individuals vary in their capacity to acquire resources relative to their allocation strategy (see also Reznick 2000). This model shows that when individuals vary little in their resource acquisition but vary greatly in their trait allocation negative relationships between traits (trade-offs) emerge. A number of studies on insects have shown that a variety of fitness-related traits trade-off with immunity (see Table 1). For example, in male decorated crickets, *Gryllodes sigillatus*, the spermatophylax (the portion of the spermatophore that serves as a nuptial gift) decreases in size as the intensity of a parasite infestation increases (Luong and Kaya 2004) and sperm viability decreases in *Teleogryllus oceanicus* crickets after an immune-challenge with pathogenic bacteria (Simmons and Roberts 2005, Simmons 2011). Moreover,

because sperm production is costly (Dewsbury 1982, Nakatsuru & Kramer 1982, Olsson et al 1997, Kelly and Jennions 2011, Scharf et al 2012), immune function sometimes declines as reproductive demands increase. In another example, male *G. sigillatus* that were forced to produce more spermatophores tended to invest less in immunocompetence, measured as phenoloxidase activity, implant melanization, and lytic activity (Kerr et al 2010). Adamo et al (2001) found that in the Texas field cricket (*Gryllus texensis)* immunocompetence, phenoloxidase activity and susceptibility to bacterial infection, decreases in reproducing males (but might increase in reproducing females). In the Japanese damselfly, *Matrona basilaris japonica* Fester, immunity and reproduction are negatively correlated so immune function (i.e. encapsulation) decreases as reproductive activity (i.e. copulation in males and oviposition in females) increases (Siva-Jothy et al 1998).

van Noordwijk and de Jong's model also showed that when variation in allocation is greater than variation in acquisition a positive correlation between traits can emerge in some individuals. This generally means that higher quality males have more resources to spend on all traits. This is the basis of theories of parasite-mediated sexual selection because traits that increase an individual's sexual attractiveness are metabolically expensive to develop, maintain, and/or perform and so should reliably signal male quality to females (Hamilton and Zuk 1982). In other words, high metabolic costs can prevent individuals in poor condition [i.e. few resources at their disposal (Tomkins et al 2004)] from producing attractive secondary sexual characteristics without appropriating resources from other important traits (Wagner and Hoback 1999, Fisher 1915). While there are some studies demonstrating trade-offs between attractiveness and

immunity (Table 1 for examples in insects), little is known about how sexual attractiveness mediates the effect of an immune challenge on male reproduction and behavior.

Positive correlations between immunity and attractiveness can arise due to factors other than male quality. For example, Sadd et al (2006) showed that immune-challenged male mealworm beetles not only increased their immune function (PO levels) but they also increased their sexual attractiveness through increased pheromone production. The authors explained that this was likely an example of terminal investment, which is defined as increased investment in reproduction at the cost of survival (Clutton-Brock 1984). Nonetheless, a positive correlation between immune function and attractiveness emerged, which was not due to male quality per se. Therefore, biologists need to proceed with caution when interpreting data in organisms that can modulate attractiveness at the cost of survival. Animals that invest in permanent sexually attractive structures, such as large body size, cannot rapidly change the amount of resources allocated to current reproduction. For instance, a bird that invests heavily in sexually attractive plumage during development cannot change the amount of resources left for other life history traits later in life. Male mealworm beetles, on the other hand, can quickly change the amount of resources they invest in current reproduction (pheromone production) depending on their likelihood to survive for future reproduction. Kivleniece et al (2010) found that male mealworm beetles are less attractive than controls after a single parasite-like activation event of the immune system, but are more attractive than controls after repeated exposure of a parasite-like immune challenge (Kivleniece et al 2010). This shows that males may reallocate resources to invest in survival or a terminal investment depending on the intensity of infection.

Maximizing fitness often requires maximizing lifespan as well as maximizing reproduction. In addition to eliciting an immune response to pathogens, animals often aid their recovery by reducing their eating, drinking, movement, and grooming activity (Hart 1988, Kyriazakis et al 1998). These are collectively known as sickness behaviors. By reducing investment in energetically demanding behaviors, animals might reduce the impact of a trade-off between immunity and other life history traits. For example, illness-induced anorexia reduces the trade-offs between immunity and digestion in *Gryllus texensis* (Adamo et al 2010). Despite the study of sickness behavior receiving considerable attention, we know very little about how sexual attractiveness mediates the effect of an immune challenge on sickness behavior.

Gryllus crickets are an excellent model system to investigate the trade-offs between immunity and attractiveness. Crickets have a short lifespan (Jacot et al 2004), male reproductive effort is easily measured (Gress and Kelly 2011), and, because there is no parental care and only one breeding season (Cade and Cade 1992), resources crickets reallocate from immunity to reproduction might have significant positive effects on reproductive fitness (Adamo et al 2001). The relationships between reproduction and immunity are well studied in crickets making them a useful model system for disentangling these relationships (Luong and Kaya 2005, Tregenza et al 2006, Shoemaker and Adamo 2007, Simmons 2011). On the other hand, there is considerable variation among and within species as to what females find attractive and there are many factors involved in attractiveness that are not well studied and/or have equivocal evidence: body size, pheromones, behavior, etc. There are a few attributes of attractiveness that are well-studied in *Gryllus*, for instance, female crickets prefer males with calling songs that have high chirp rates, long duration, and loud amplitude, which are energetically expensive to produce (Hoback and

Wagner 1997; also in *Physalaemus* Bucher et al 1982). Therefore, only males in good condition have the energetic resources available to frequently produce attractive calls (Wagner and Hoback 1999) and are more likely to attract a female and entice her to mate. In ground crickets, *Allonemobius socius*, male calling behavior changes (the time between pulses within chirps is longer) when males are immune challenged (Fedorka and Mousseau 2006). However, the effects of immune challenges on total reproductive effort, particularly reproductive behaviors, and how they correlate with attractiveness are still largely unknown.

In this study, I investigate whether male attractiveness mediates trade-offs between reproduction and immunity in the Texas field cricket (*Gryllus texensis*: Orthoptera, Gryllidae). I used controlled experiments in the laboratory to test Hypothesis 1: that attractive males are better able to acquire and assimilate nutritional resources compared to unattractive males (see Hypothesis 1 in Table 2). Under Hypothesis 1, I predict 1) that an immune-challenge will cause more sickness behaviors in unattractive males but not in attractive males, 2) that an immune-challenge will cause unattractive males to increase calling behavior, and 3) that attractive males will have better body condition than unattractive males (Wagner and Hoback 1999). An alternative hypothesis (see Hypothesis 2 in Table 2) poses that attractive and unattractive males have similar resource pools but vary in how they allocate those resources. Under Hypothesis 2, I predict: 1) that an immune-challenge will cause more sickness behaviors in attractive but not in unattractive males, 2) that an immune-challenge will cause attractive males to increase calling behavior, and 3) that they do not differ in their body condition. To test these predictions, I immune-challenged sexually attractive and unattractive male crickets with an immune elicitor,

lipopolysaccharide (LPS), and then examined the effects on a variety of fitness-related behaviors and the presence of a spermatophore.

CHAPTER 2

EXPERIMENT

MATERIALS AND METHODS

Study Species and Laboratory Maintenance

Gryllus texensis is a field cricket ranging from western Texas to western Florida and from Oklahoma to Mexico (Cade and Otte 2000). *G. texensis* males attract females to their location with a calling song and entice them to mate with a courtship song (Fitzpatrick and Gray 2001; Gray and Eckhardt 2001). Copulation requires females to voluntarily mount males, after which the male vibrates his cerci while passing a spermatophore to the female. The spermatophores only transfer ejaculate and do not have a spermatophylax (food gift) attached. Males cannot force copulation. Spermatophore transfer generally takes 5-6 seconds (personal observation).

Crickets were lab-reared descendants of individuals originally caught in Austin, TX (USA) in 2012 and 2013. The laboratory colony of crickets was reared in large communal bins (73x41x46 cm) until their penultimate instar, after which they were kept in large sex-specific communal bins. Upon eclosion to adulthood, crickets were each transferred to individual 10 cm deli cups. All crickets were housed in an environmentally controlled room (27° C, 12:12 h light:dark cycle, 80% relative humidity) and were supplied with cotton-plugged water vials and dry cat food (Special Kitty Premium Cat Food) ad libitum. Crickets were used in experiments 10-14 d post-eclosion to ensure they were sexually mature.

Morphological Traits

Before the experiment began, all males were weighed on a Denver Instruments TP-64 digital balance and photographed to measure pronotum length (the distance from the anterior to posterior edges of the pronotum at the midline) as a proxy for body size. After the behavioral trial, all males were frozen, dissected, and their testes removed. Testes were put on a bridge slide (a microscope slide with 4 cover slips glued to each side so that specimens can be placed in the center and pressed to the same thickness) and photographed. The area touching the coverslip was measured using ImageJ (National Institute of Health). All photographs were taken using a Leica S6D stereomicroscope and Leica Application Suite image analysis software (Leica Microsystems Inc., Buffalo Grove, IL, USA).

Quantifying Attractiveness

I assessed the attractiveness of each experimental male by using the tournament-based protocol of Shackleton et al (2005), which uses latency to mate to compare female preference for different males (see A1). I opted for this method because the female preference of conspecifics takes the whole male into account rather than assigning attractiveness on a single measureable trait, such as body size or courtship calling, which does not encompass all the traits that females might find attractive. Latency to mate is a common measure for attractiveness (Ritchie et al 1999, Head et al 2005, Shackleton et al 2005, Bussiere et al 2006). All trials were conducted under red light (25 watt, 125 volt, Red Sylvania bulb) to minimize observer disturbance. Two rounds of trials were done on two consecutive days and all experimental activity started as the light cycle switched from light to dark. Twelve males were put in individual 10 cm diameter deli cups with white paper lining the bottom (for traction) and a randomly selected female. For

logistical reasons, the lining was not changed between rounds that occurred on the same day (rounds 1 and 2 and rounds 3 and 4) and remained with the same male. Whichever male was mounted (but not mated) first was ranked 1, and the last male mounted was ranked 12 (if multiple males did not mate within 60 minutes they shared the average of the remaining ranks). A female must have remained on top of a male while he vibrated his cerci for 3 seconds to be considered a 'mounting' and they were separated before the male transferred a spermatophore to the female. In the second round females swapped places with the female paired with the male of corresponding opposite attractiveness, so the female paired with the highest-ranking male and the female paired with the lowest-ranking male in the first round switched males for the second round. This helped control for individual female willingness to mate. After the second round, females were returned to their individual deli cups with a white paper lining for the next 22 hours, which were later used to build the behavior arenas. On the second day, the same females were randomly paired with a male they had not been paired with previously for the third trial. For the fourth trial, females swapped places with the female paired with the male of corresponding opposite attractiveness in the third trial. Again, a fresh piece of white paper was used to line the bottom of the female deli cups for the next 22 hours for use in the behavioral arenas.

Ranks from all four trials were summed for the males. Those with the four lowest sums were considered attractive while those with the four highest sums were considered unattractive. The four mid-ranking males were discarded from the behavior study. Seven rounds of attractiveness trials produced the 28 attractive and 28 unattractive males that were used in the behavior study.

Administering an Immune Challenge

After the males' attractiveness was determined, I immune-challenged the attractive and unattractive males by following the protocol of Shoemaker and Adamo (2007). This third day of the experiment was also started when the light cycle changed from light to dark. Timing was staggered so that 2 crickets were anesthetized and injected per 10 minutes. First, I cold anesthetized two crickets simultaneously by placing them in a clean 10 cm deli cup pressed in ice for 10 minutes. Males were injected using a microcapillary needle (needles were used only once) along the left pleural region of their abdomen. Microcapillary needles were made in a Flaming/Brown Micropipette Puller (Sutter Instrument Co. model P-97, program 27) with Kwik-Fil's borosilicate glass capillaries. Fourteen attractive males and 14 unattractive males were injected with 5μL of saline (phosphate-buffered saline, Sigma-Aldrich), and 14 attractive males and 14 unattractive males were injected with 100μg of Lippopolysacharides from *Serratia marcescens* (LPS, Sigma-Aldrich), dissolved in 5μL of saline. *S. marcescens* is a common soil microbe where *G. texensis* occur and is potentially a natural pathogen (Stevenson 1959). LPS stimulates the cricket's immune system to protect the body from what it 'interprets' as a bacterial infection; however, this method does not induce disease symptoms. Recordings began one hour after males were injected to allow the LPS to take effect and lasted 5 hours (see below). This timeframe for challenging and measuring males' behavior falls within the active period of the LPS injection (Yusuke et al 1994).

Recording Behavior

Immediately following injection of LPS or saline, males were put into a 9" x 6" x 6 ½" arena (Exo Terra Faunarium). The ventilated section of the lids were removed and replaced with

a mesh screen. The arena contained a paper shelter (made from an Oxford 3"x5" index card cut into 4 strips), one piece of Special Kitty cat chow, and a water vial all fixed with hot glue to a white paper lining the bottom (Adler and Uebel 1985, Silverman 1986). Two randomly-chosen papers from female deli cups were placed under the arena lining to introduce the pheromones of reproductively mature females and elicit reproductive behaviors. Males were given one hour to acclimate to the arena after which a spermatophore was taken by using forceps to move the subgenital plate downward and gently lift the spermatophore from the genital chamber. This spermatophore was discarded and forced males to produce a new spermatophore during the immune challenge or continue without the possibility of mating. The males were then recorded for 5 h with a Canon Vixia HFG10 HD camcorder. Crickets did not all share the same start time of the acclimatizing hour and behavior recordings. They were staggered so that 2 crickets were processed per 10 minutes and the first and last crickets only had a difference in start time of 30 minutes. The behavioral trials were filmed using four CMVision IR200-940 IR Illuminators (18 watt, infrared lamps) and one red light for the observer to see. During the trials, I also directed a microphone (Dynex USB, DX-USBMIC13) toward each male for 5 seconds every five minutes to assess male calling. For each male, I held the microphone above the screen top of its arena, within 9 inches of the male. I used QuickTime Player (version 10.2, Apple Inc.) to visualize any sound detected by the microphone and documented whether or not an individual was calling. Calling males were assigned a value of 1 whereas males that were not calling were assigned a value of 0 for each 5-minute interval. This procedure allowed me to estimate the individual calling effort of each male in real time. In addition, I noted times during the trials when males ate. At the end of the five-hour behavioral trials, males were checked for the presence of a spermatophore and videos were analyzed for movement and hiding behaviors using the program

EthoVision (XT 8.5, Noldus). To assess how the behaviors changed over time, I binned all of the data into half-hour bins. For movement, I quantified both time spent moving and time spent stationary/hidden each half hour. For hiding, I quantified both time spent in the shelter and time spent exposed per half hour. I quantified calling as the number of occasions that I observed males calling per half hour. Because I only used the microphone to assess calling once every 5 minutes, the maximum number of times that a male could call in each half-hour bin was 6.

Statistical Analysis

I used pronotum length because it is a common proxy for body size in orthopterans (Gwynne 1988, Zuk 1988, Larson et al 2013). To examine body condition I used the scaled mass index method described by Kelly et al (2014). I then used a T test to compare the scaled mass index of attractive and unattractive males (N=62). I used a T test to determine if the attractive and unattractive groups differed in body size (N=62). I then used an ANCOVA (with body size entered as a covariate) to determine if testis size differed between attractive and unattractive males (N=62).

I used binomial generalized linear models to determine if spermatophore presence was correlated with body size or attractiveness before males were injected and again after the injection to determine if spermatophore presence changed with an immune challenge ($N = 56$).

I used a binomial regression to determine if the proportion of calling in attractive and unattractive males was affected by an immune challenge ($N = 56$).

I used a linear model to determine if the total number of eating events was affected in attractive and unattractive males by an immune challenge (N=56).

I used a linear mixed effect model with denominator degrees of freedom Kenward-Roger adjusted to determine if the total distance moved per ½ hour was affected in attractive and unattractive males by an immune challenge and the time that passed since injection.

I used a repeated measures anova with denominator degrees of freedom Kenward-Roger adjusted to determine if the proportion of time spent hidden per ½ hour was affected in attractive and unattractive males by an immune challenge and the time that passed since injection.

RESULTS

Attractiveness Groups

The attractive and unattractive groups did not differ in pronotum length ($T=$ -0.537, $d.f.=$ 57.359, $P = 0.594$) or in body condition ($T=$ -0.455, $d.f. = 59.917$, $P = 0.651$, Figure 1). Testis size did not differ between attractive and unattractive groups ($F_{1,75} = 0.232$, $P = 0.632$).

Spermatophore Presence

Spermatophore presence was not correlated with either attractiveness ($\chi^2 = 0.00$, $d.f. = 1$, $P = 1.00$) or body size ($\chi^2 = $ -0.032, $d.f. = 1$, $P = 0.974$) prior to the immune challenge (Table 4). Moreover, after males were injected with either saline or LPS spermatophore presence was not significantly affected by injection treatment ($\chi^2 = $ -0.370, $d.f. = 1$, $P = 0.699$), attractiveness ($\chi^2 = 0.003$, $d.f. = 1$, $P = 0.998$), or body size ($\chi^2 = 0.475$, $d.f. = 1$, $P = 0.635$).

Calling

There was a significant interaction between attractiveness and injection treatment on calling frequency ($\chi^2 = 6.009$, $d.f. = 1$, $P = 1.86e\text{-}09$). Attractive males called significantly more frequently when injected with LPS than saline. Unattractive males called significantly less frequently when injected with LPS than saline (Figure 2).

Feeding Events

There was a significant interaction between injection treatment and attractiveness (Males decreased the number of eating events as time since injection increased (χ^2 = 12.071, *d.f.* = 1, *P* = 0.022, see A3). There was a trend (non-significant) for attractive males to eating more frequently when injected with saline than LPS, whereas unattractive males (also non-significant) tended to eat more frequently when injected with LPS than saline (Table 3).

Distance Traveled

All males increased the distance they traveled as the time since injection increased ($F_{1, 491.88}$ = 19.998, *P* = 9.636e-6, See A5). There were no significant differences in distance travelled between attractiveness ($F_{1, 52.99}$ = 1.52, *P* = 0.223) or injection treatment ($F_{1, 52.99}$ = 0.084, *P* = 0.774).

Hiding

There was a significant interaction between treatment and time since injection (χ^2 = 2.255, *d.f.* = 1, *P* = 0.024, Figure 3). Unattractive males and attractive males injected with LPS hid the same amount throughout the night. Attractive males injected with saline hid less early on but then hid as much as the others later on.

DISCUSSION

Being sexually attractive has obvious benefits for animals, allowing increased
reproductive opportunities (Andersson 1994). However, traits that make individuals more
sexually attractive are often energetically expensive and may incur additional costs such as
increased threat of predation (Andersson 1994, Zuk and Kolluru 1998). Individuals might
increase their sexual attractiveness relative to conspecifics in one of two ways. First, attractive
individuals might be better at acquiring resources than conspecifics giving them increased
reserves to allocate toward all life-history traits (Hypothesis 1). Sexually attractive traits would
then be honest signals of overall quality. Alternatively, attractive individuals might preferentially
allocate their limited resources toward attractive traits at the expensive of other life-history traits,
such as maintenance through immunity (Hypothesis 2). The behavioral responses of attractive
and unattractive male *G. texensis* (as selected by females) in response to an immune challenge
that I observed suggest that development of attractive traits comes at the expense of immune
function, and thus support Hypothesis 2.

If attractive males acquire and assimilate more resources than unattractive males, as
proposed by Hypothesis 1, attractive males should invest more heavily in all life history traits
than unattractive males. Under this hypothesis, attractive males are predicted to be in better
condition, have larger testes, and have a spermatophore prepared more frequently (i.e., shorter
refractory time) than unattractive males (Simmons 1986A; Simmons 1988; Andersson 1994;
Brown et al 1996). In addition, attractive males are predicted to mount an immune defense with
relatively little cost to their other life-history traits. Thus, when immune challenged, attractive
males should not significantly alter their calling, eating, hiding, or movement in response to an

immune challenge, whereas unattractive males are predicted to display significantly altered behaviors and perhaps terminally invest. However, I observed that male attractiveness was not correlated with body condition, testis size, or refractory period. Furthermore, contrary to the predictions of Hypothesis 1, attractive males altered their calling effort and displayed sickness anorexia in response to an immune challenge while unattractive males did not. These results allow me to reject Hypothesis 1.

Under Hypothesis 2, all males are similarly able to acquire/assimilate resources, but males differentially allocate resources to attractiveness. Body condition is predicted to be similar between attractive and unattractive males under this hypothesis, as I observed. In addition, Hypothesis 2 predicts that attractive males will have reduced resources available to mount an immune defense, and thus be more negatively impacted by an immune challenge than unattractive males. If resource availability in attractive males is insufficient to survive infection, an immune challenge might also force attractive males to produce a terminal investment. As predicted by Hypothesis 2, attractive males in my experiments altered their behavior in response to an immune challenge while unattractive males did not. Moreover, the behavioral changes made by attractive males suggest that they exhibited a terminal investment: attractive males increased their calling efforts and decreasing feeding in response to an immune challenge. If, in fact, attractive males are in such good condition and have accumulated so many resources that they do not need to eat after an immune challenge they should eat the same amount as males that were injected with saline. These behavioral changes could increase short-term reproductive

output, but at the expense of surviving the immune challenge. Taken together, these results support the predictions of Hypothesis 2.

Even though many of my observations support Hypothesis 2, distance traveled, time spent hiding, and refractory time (when injected with LPS) did not match my *a priori* predictions of either hypothesis. Distance traveled and time spent mobile were predicted to increase while time spent hiding was predicted to decrease as a result of mate searching in males displaying a terminal investment (unattractive LPS males in Hypothesis 1 and attractive LPS males in Hypothesis 2). I predicted that these behaviors would remain unaltered in males that had ample resources. However, I observed no differences in these behaviors for either attractive or unattractive males. In nature, *Gryllus* males generally remain close to burrows when calling (personal observation, Hissman 1990). Thus, it is plausible that increased hiding could facilitate calling and result from a terminal investment strategy. If so, increased hiding by attractive LPS males could support Hypothesis 2. Further work on the interaction between calling, hiding, and immunocompetence are needed to tease apart these possibilities.

Since the fixed attributes measured in this study (body and testis size) did not contribute to attractiveness, perhaps males can rapidly change resource allocation to and from attractive traits (e.g. behavior or pheromone production). If so, attractiveness might have low repeatability over their reproductive life span. Because I observed consistent differences in behavior between attractive and unattractive males for 3 days, attractiveness is likely repeatable over this time-

span, but might not be over longer time-spans. It would be informative to conduct a similar study in a species that cannot change their sexual attractiveness quickly (i.e. the plumage in the male long-tailed widowbird, Andersson 1982).

There are several ways one could improve upon this study for future work. For example, it would be useful to include a "control" group. Males injected with LPS and saline should be compared to males that did not undergo an injection, as the injection itself might have instigated an immune response. Sperm counts and sperm viability of attractive and unattractive males could then be compared in all 3 of these groups. Male attractiveness should also be tested multiple times throughout male reproductive life span to better understand whether it is fixed or transitory. Finally, this experiment was done with ad libitum food, which might skew the results away from what would be found naturally. This experiment could very possibly have a very different out come if males were put on a low quality or low quantity diet (Kelly and Tawes 2013).

My results suggest that variation in attractiveness among male Texas field crickets results from differential allocation of similar resource quantities. Because of this, attractive males were more negatively impacted by an immune challenge than unattractive males. In this system, attractiveness is not an honest signal of all-around quality and males appear to be under selection to express attractive traits at the expense of other traits, particularly immunity.

REFERENCES

Adamo, S. A. 1999. Evidence for adaptive changes in egg laying in crickets exposed to bacteria and parasites. Animal Behaviour. 57:117-124.

Adamo, S. A., Jensen, Younger. 2001. Changes in lifetime immunocompetence in male and female *Gryllus texensis* (formerly *G. integer*): Trade-offs between immunity and reproduction. Animal Behaviour. 62: 417-425.

Adamo, S. A., Bartlett, Le, Spencer, Sullivan. 2010. Illness-induced anorexia may reduce trade-offs between digestion and immune function. Animal Behaviour. 79 (1): 3-10.

Adler, V. E., Uebel. 1985. Effect of a formulation of neem extract on six species of cockroaches (Orthoptera: Blaberidae, Blattidae and Blattellidae). Phytoparasitica. 13(1): 3-8.

Andersson, M. 1994. Sexual selection. Princeton Press, New Jersey.

Andersson, M. 1982. Female choice selects for extreme tail length in a widowbird. Nature. 299: 818-820.

Brown, W. D., Wideman, Andrade, Mason, Gwynne. 1996. Female choice for an indicator of male size in the song of the black-horned tree cricket, *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). Evolution. 50 (6): 2400-2411.

Bucher, T. L., Ryan, Bartholomew. 1982. Oxygen consumption during resting, calling, and nest building in the frog *Physalaemus pustulosus*. Physiological Zoology. 55 (1): 10-22.

Bussiere, L. F., Hunt, Jennions, Brooks. 2006. Sexual conflict and cryptic female choice in the black field cricket, *Telleogryllus commodus*. Evolution. 60 (4): 792-800.

Cade, W. H., Cade. 1992. Male mating success, calling and searching behaviour at high and low densities in the field cricket, *Gryllus integer*. Animal Behaviour. 43: 49-56

Cade, W. H., Otte. 2000. *Gryllus texensis* n. sp.: A widely studied field cricket (Orthoptera; Gryllidae) from the southern United States. Transactions of the American Entomological Society. 126 (1): 117-123.

Clutton-Brock, T. H. 1984. Reproductive effort and terminal investment in iteroparous animals. American Naturalist. 123 (2): 212-229.

Dewsbury, D. A. 1982. Ejaculate cost and male choice. American Society of Naturalists. 119 (5): 601-610.

Dowling, D. K. and Simmons. 2012. Ejaculate economics: Testing the effects of male sexual history on the trade-off between sperm and immune function in australian crickets. PLoS ONE. 7 (1): 1-6.

Drayton, J.M., Boeke, Jennions. 2013. Immune challenge and pre- and post-copulatory female choice in the cricket *Teleogryllus commodus*. Journal of Insect Behavior. 26: 176-190.

Fedorka, K. M., Mousseau. 2006. Immune system activation affects male sexual signal and reproductive potential in crickets. Behavioral Ecology. 18 (1): 231-235.

Fisher, R. A. 1915. Frequency distribution of the values of the correlation coefficient in samples from an indefinitely large population. Biometrika. 10 (4): 507-521.

Fitzpatrick, M. J., Gray. 2001. Divergence betwee courtship songs of the field crickets *Gryllus texensis* and *Gryllus rubens* (Orthoptera, Gryllidae). Ethology. 107: 1075-1085.

Gershman, S. N., Barnett, Pettinger, Weddle, Hunt, Sakaluk. Give 'til it hurts: trade-offs between immunity and male reproductive effort in the decorated cricket, *Gryllodes sigillatus*. Journal of Evolutionary Biology. 23 (4): 829-839.

Gray, D. A., & Eckhardt. 2001. Is cricket courtship song condition dependent? Animal Behaviour. 62 (5): 871-877.

Gress, B., Kelly. 2011. Is sperm viability independent of ejaculate size in the house cricket (*Acheta domesticus*)? Canadian Journal of Zoology. 1236: 1231–1236.

Gwynne, D. T. 1988. Courtship feeding and the fitness of female katydids (Orthoptera: Tettigoniidae). Evolution. 42 (3): 545-555.

Hart, B. L. 1988. Biological Basis of the behavior of sick animals. Neuroscience and Biobehavioral Reviews. 12: 123-137.

Hamilton, W. D., Zuk. 1982. Heritable true fitness and bright birds: A role for parasites. Science. 218 (4570): 384-387.

Head, M. L., Hunt, Jennions, Brooks. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. PLoS Biology. 3 (2): 1-6.

Hissman, K. 1990. Strategies of mate finding in the European field cricket (Gryllus campestris) at different population densities: a field study. Ecological Entomology. 15 (3): 281-291.

Hoback, W. W., Wagner. 1997. The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps.* Physiological Entomology. 22: 286-290.

Jacot, A., Scheuber, Brinkhof. 2004. Costs of an immune response on sexual display and longevity in field crickets. Evolution. 58 (10): 2280-2286.

Jones, J. S. 1990. Living fast and dying young. Nature. 348: 288-289.

Kelly, C. D., Jennions. 2011. Sexual selection and sperm quantity: meta-analyses of strategic ejaculation. Biological Reviews. 86 (4): 863-884.

Kelly, C. D., Tawes. 2013. Sex-specific effect of juvenile diet on adult disease resistance in a field cricket. PLoS ONE. 8 (4): 1-7.

Kelly, C. D., Tawes, Worthington. 2014. Evaluating indices of body condition in two cricket species. Ecology and Evolution. doi: 10.1002/ece3.1257

Kerr, A. M., Gershman, and Sakaluk. 2010. Experimentally induced spermatophore production and immune responses reveal a trade-off in crickets. Behavioral Ecology. doi:10.1093/beheco/arq035

Kivlenience, I., Krams, Daukste, Krama, Rantala. 2010. Sexual attractiveness of immune-challenged male mealworm beetles suggests terminal investment in reproduction. Animal Behavior. 80 (6): 1015-1021.

Krams, I., Daukste, Kivlenience, Krama, Rantala, Ramey, Sausa. 2011. Female choice reveals terminal investment in male mealworm beetles, *Tenebio molitor*, after a repeated activation of the immune system. Journal of Insect Science. 11 (56): 1-14.

Kyriazakis, I., Tolkamp, Hutchings. 1998. Towards a functional explanation for the occurance of anorexia during parasitic infections. Animal Behaviour. 56 (2): 265-274.

Larson, E. L., Becker, Bondra, Harrison. 2013. Structure of a mosaic hybrid zone between the field crickets *Gryllus firmus* and *G. pennsylcanicus*. Ecology and Evolution. 3 (4): 985-1002.

Lawniczak, M., Barnes, Linklater, Boone, Wigby, Chapman. 2007. Mating and immunity in invertebrates. Trends in Ecology & Evolution. 22 (1): 48-55.

Leman, J. C., Weddle, Gershman, Kerr, Ower, St John, Vogel, Sakaluk. 2009. Lovesick: immunological costs of mating to male sagebrush crickets. Journal of Evolutionary Biology. 22 (1): 163-171.

Luong, L., Kaya. 2005. Sexually transmitted parasites and host mating behavior in the decorated cricket. Behavioral Ecology. 16 (4): 794-799.

McNamara, K. B., Wedell, Simmons. 2013. Experimental evolution reveals trade-offs between mating and immunity. Biology Letters. 9 (4): 20130262.

McKean, K. A., Nunney. 2001. Increased sexual activity reduces male immune function in *Drosophila melanogaster*. Proceeding of the National Academy of Sciences of the United States of America. 98 (14): 7904-7909.

Nakatsuru, K. Kramer. 1982. Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). Science. 216 (4547): 753-755.

van Noordwijk, A. J., de Jong. 1986. Acquisition and allocation of resources: Their influence on variation in life history tactics. The American Naturalist. 128 (1): 137-142.

Olsson, M., Madsen, Shine. 1997. Is sperm really so cheap? Costs of reproduction in male adders, *Vipera berus*. Proceedings of the Royal Society of London. B. 264 (1380): 455-459.

Reznick, D., Nunney, Tessier. 2000. Big houses, big cars, superfleas and the costs of reproduction. Nature. 15 (10): 421-425.

Ritchie, M. G., Halshey, Gleason. 1999. Drosophila song as a species-specific mating signal and the behavioural importance of Kyriacou & Hall cycles in *D. melanogaster* song. Animal Behaviour. 58: 649-657.

Roff, D. A. 1993. The evolution of life histories: Theory and analysis. Chapman & Hall, New York.

Sadd, B., Holman, Armitage, Lock, Marland, Sivy-Jothy. 2006. Modulation of sexual signaling by immune challenged male mealworm beetles (*Tenebrio molitor*, L.): Evidence for terminal investment and dishonesty. Journal of Evolutionary Biology. 19 (2): 321-325.

Scharf, I., Peter, Martin. 2012. Reproductive trade-offs and direct costs for males in arthropods. Evolutionary Biology. DOI 10.1007/s11692-012-9213-4

Shackleton, M. A., Jennions, Hunt. 2005. Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: The effectiveness of no-choice tests. Behavioral Ecology and Sociobiology. 58 (1): 1-8.

Shoemaker, K. L., Adamo. 2007. Adult female crickets, *Gryllus texensis*, maintain reproductive output after repeated immune challenges. Physiological Entomology. 32 (2): 113–120.

Shoemaker, K. L., Parsons, Adamo. 2006. Egg-laying behavior following infection in the cricket *Gryllus texensis*. Canadian Journal of Zoology. 84 (3): 412-418.

Silverman, J. 1986. Adult German cockroach (Orthoptera: Blattellidae) feeding and drinking behavior as a function of density and harborage-to-resource distance. Environmental Entomology. 15: 198-204.

Simmons, L. W. 1986 A. Female choice in the field cricket *Gryllus bimaculatus* (De Greer). Animal Behaviour. 34: 1463-1470.

Simmons, L. W. 1986 B. Inter-male competition and mating success in the field cricket, *Gryllus bimaculatus* (de Geer). Animal Behviour. 34: 567-579.

Simmons, L. W. 1987. Sperm competition as a mechanism of female choice in the field cricket, *Gryllus bimaculatus*. Behavioral Ecology and Sociobiology. 21 (3): 197-202.

Simmons, L. W. 1988. Male size, mating potential and lifetime reproductive success in the field cricket, Gryllus bimaculatus (De Greer). Animal Behaviour. 36: 372-379.

Simmons, L. W. 2011. Resource allocation trade-off between sperm quality and immunity in the field cricket, *Teleogryllus oceanicus*. Behavioral Ecology. 23 (1): 168-173.

Simmons, L. W., Roberts. 2005. Bacterial immunity traded for sperm viability in male crickets. Science. 309 (5743): 2031-2031.

Siva-Jothy, M. T., Tsubaki, Hooper. 1998. Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. Physiological Entomology. 23: 274-277.

Stahlschmidt, Z. R., Rollinson, Acker, Adamo. 2013. Are all eggs created equal? Food availability and the fitness trade-off between reproduction and immunity. Functional Ecology. 27 (3): 800-806.

Stevenson, J. P. 1959. Epizootiology of a disease of the desert locust, *Schistocerca gregaria* (Forskal), caused by nonchromogenic strains of *Serratia marcescens* Bizio. Journal of Insect Pathology. 1: 232-244.

Stearns, S. C. 1992. The Evolution of Life Histories. Oxford, New York.

Tomkins, J. L., Radwan, Kotiaho, Tregenza. 2004. Genic capture and resolving the lek paradox. Trends in Ecology & Evolution. 19 (6): 323-328.

Tregenza, T., Simmons, Wedell, Zuk. 2006. Female preference for male courtship song and its role as a signal of immune function and condition. Animal Behaviour. 72: 809-818.

Vainikka, A., Rantala, Seppala, Suhonen. 2007. Do male mealworm beetles, *Tenebrio molitor,* sustain the honesty of pheromone signals under immune challenge? Acta Ethologica. 10 (2): 63-72.

Wagner, W. E., Hoback. 1999. Nutritional effects on male calling behavior in the variable field cricket. Animal Behaviour. 57 (1): 89-95.

Yusuke, K., Motoi, Taniai, Kadono-Okuda, Hiramatsu, Yamakawa. 1994. Clearance of lipopolysaccharide in hemolymph of the silkworm *Bombyx mori*: Its role in the

termination of cecropin mRNA induction. Insect Biochemisry and Molecular Biology. 24 (6): 539-545.

Zuk, M. 1988. Parasite load, body size, and age of wild-caught male field crickets (Orthoptera: Gryllidae): Effects on sexual selection. Evolution. 42 (5): 969-976.

Zuk, M., Kolluru. 1998. Exploitation of sexual signals by predators and parasititoids. The Quarterly Review of Biology. 73 (4): 415-438.

Table 1: Studies examining the relationship between immune response and attractiveness, behavior, and/or reproduction in insects.

Paper	Species	Trade-off between Immunity and:	Finding
Jacot et al 2004	*Gryllus campestris*	Attractiveness	Immune challenges cause a decrease in attractiveness and longevity
Fedorka and Mousseau 2006	Allonembius socius	Attractiveness	Immune challenge had a negative effect on male calling song, attractiveness, and nuptial gift.
Drayton et al 2013	Teleogryllus commodus	Attractiveness	No change in pre- or post-copulatory male attractiveness with immune-challenge
Sadd et al 2006	*Tenebrio molitor*	Attractiveness	Immune challenged males increase reproductive effort (pheromone production)
Vainikka et al 2007	*Tenebrio molitor*	Attractiveness	Immune challenges did not affect attractiveness of male pheromones
Adamo 1999	*Acheta domesticus*	Reproduction	Activation of some immune responses causes females to increase egg laying
Adamo et al 2001	*Gryllus texensis*	Reproduction	Immunocompetence decreases in males as they begin reproductive behaviors Immunocompetence might increase in reproductively active females.
Shoemaker and Adamo 2007	*Gryllus texensis*	Reproduction	When resources are provided *ad libitum* number and weight of eggs laid is not affected by an immune-challenge Larger immune challenges lead to decreased female longevity and egg weight
Stahlschmidt et al 2013	Gryllus texensis	Reproduction	Chronically immune challenged females exhibited lower fecundity but higher egg protein
Gershman et al 2010	Gryllodes sigillatus	Reproduction	Males with larger spermatophalaxes had lower lytic activity
Kerr et al 2010	Gryllodes sigillatus	Reproduction	When injected with LPS spermatophore size decreased and when they produced more spermatophores immnocompetence, implant darkness, PO activity, and lytic activity, decreased
Simmons and Roberts 2005	Teleogryllus oceanicus	Reproduction	Sperm viability decreases with increased lysozyme activity
Tregenza et al 2006	Teleogryllus oceanicus	Reproduction	Females preferred the songs of males with high immune responses.
Siva-Jothy et al 1998	*Matrona basilaris japonica* Fester	Reproduction	Immune function (encapsulation) decreases after oviposition in females and shows a decreasing trend after copulation in males
McNamara et al 2013	Plodia interpunctella	Reproduction	Increased mating rate, not sperm competition, decreased PO activity
Dowling and Simmons 2012	Teleogryllus oceanicus	Reproduction and behavior	Increased courtship behaviors decreased sperm viability over time
McKean and Nunney 2001	*Drosophila melanogaster*	Reproduction and behavior	Increased sexual activity decreases male immune function

Table 1 Continued

Paper	Species	Trade-off between Immunity and	Finding
Kivleniece et al 2010	Tenebrio molitor	Attractiveness and Terminal Investment	Attractiveness of males decreased with a single parasite-like immune challenge, but increased with multiple parasite-like immune challenges
Krams et al 2011	*Tenebrio molitor*	Attractiveness and Terminal Investment and Behavior	Males exposed to: a) 1 parasite-like immune challenge decreased male attractiveness and movement. b) 2 parasite-like immune challenges increased attractiveness but decreased movement and survival. c) 3 parasite-like immune challenges males immunocompetence was lower in in more attractive males.
Leman et al 2009	Cyphoderris strepitans	Attractiveness and behavior	An Immune challenge decrease the time males spent calling to females

Table 2: A comparison of the predicted data for how attractive and unattractive male *Gryllus texensis* differ and will change from a healthy to immune challenged state for alternative hypotheses and the observed data. Under Spermatophore, "Control" is the same group of males before the injection. Behavioral variables were recorded as the difference between males injected LPS compared to males injected with saline.

		Hypotheses					
		Hypothesis 1: Attractive males have greater quantities of resources than unattractive males, and allocate those resources similarly among life-history traits.		Hypothesis 2: Attractive and unattractive males have access to the same quantity of resources, but differentially allocate those resources among life-history traits.			
		Predictions				Observed Data	
		Attractive males show less pronounced trade-offs. Only unattractive males produce a terminal investment.		Attractive males show more pronounced trade-offs. Only attractive males produce a terminal investment.			
		Attractive Males	Unattractive Males	Attractive Males	Unattractive Males	Attractive Males	Unattractive Males
Body Condition		Greater Body Condition	Lesser Body Condition	No Difference	No Difference	No Difference	No Difference
Testes Size		Larger than Unattractive	Smaller than Attractive	Larger than Unattractive OR No Difference	Smaller than Attractive OR No Difference	No Difference	No Difference
Spermatophore Do attractive or unattractive groups produce more spermatophores	Pre-injection	More than Unattractive	Less than Attractive	More than Unattractive	Less than Attractive	Same as Unattractive	Same as Attractive
	Pre-injection to Saline	No Difference	No Difference	No Difference	No Difference	No Difference	No Difference
	Saline to LPS	No Difference	More than Attractive	More than Unattractive	No Difference	No Difference	No Difference
Reproductive Behavior Saline to LPS	Calling Effort	No Difference	Increase	Increase	No Difference	Increase	No Difference
Other Fitness Behaviors Saline to LPS	Eating Events	No Difference	Decrease	Decrease	No Difference	Decrease	No Difference
	Distance Traveled	No Difference	Increase	Increase	No Difference	No Difference	No Difference
	Time Spent Hiding	No Difference	Decrease	Decrease	No Difference	No Difference	No Difference

Table 3: Pairwise tables for calling and eating in attractive (A) and unattractive (U) male *Gryllus texensis* injected with saline (Sal) or lipopolysaccharide (LPS). Above the diagonal are unadjusted P values **bolded** if **P <0.05** when Tukey adjusted and *italicized* if *P<0.10* when Tukey adjusted. Diagonals (grey) are the least square means ± the standard error. Below the diagonal are the test statistics.

Calling

|	A Sal	A LPS	U Sal	U LPS	N=
A Sal	-2.85 ± 0.15	**<0.0001**	**<0.0001**	**0.0004**	14
A LPS	5.66	-1.82 ±0.10	0.7796	*0.0208*	14
U Sal	-5.90	-0.28	-1.78 ± 0.10	**0.0097**	14
U LPS	3.57	2.31	-2.59	-2.17 ± 0.11	14

Eating

	A Sal	A LPS	U Sal	U LPS	N=
A Sal	1.57 ± 0.41	0.0344	0.0619	0.4552	14
A LPS	-2.74	0.36 ± 0.41	0.8034	0.1710	14
U Sal	2.42	-0.52	0.50 ± 0.41	0.2626	14
U LPS	-0.9	-2.08	1.67	1.42 ± 0.41	14

Table 4: Number of attractive and unattractive male *Gryllus texensis* with spermatophores before injections and 6 hours after they were injected with saline or lipopolysaccharide (LPS).
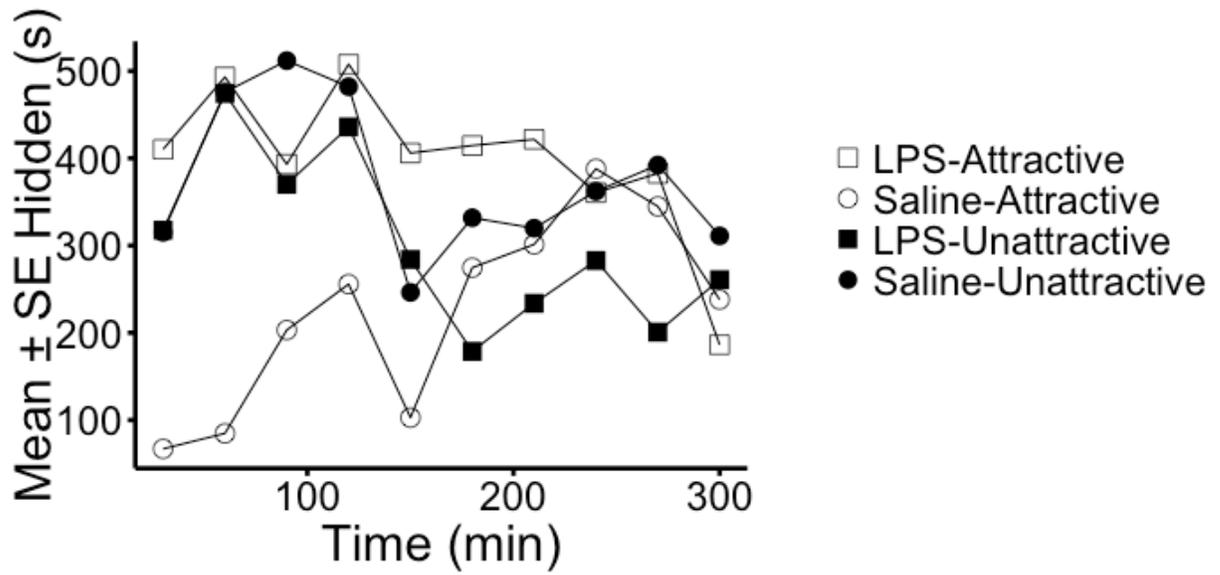
	Attractive		Unattractive	
	Spermatophore Present	Spermatophore Absent	Spermatophore Present	Spermatophore Absent
Pre-injection	18	9	18	9
Saline	10	4	8	6
LPS	8	5	10	3
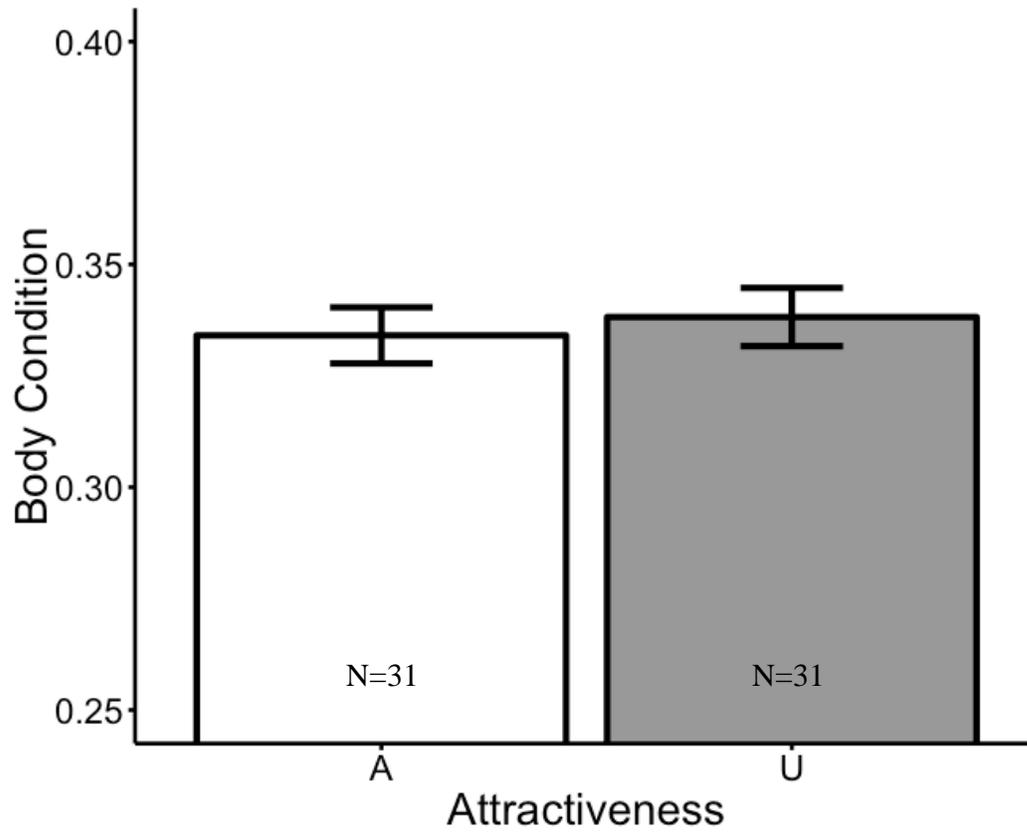
Figure 1: Mean (± 1.0 S.E.) body condition of attractive and unattractive male Texas field crickets *(Gryllus texensis)* (*T*= -0.455, *d.f.* = 59.917, *P* = 0.651).
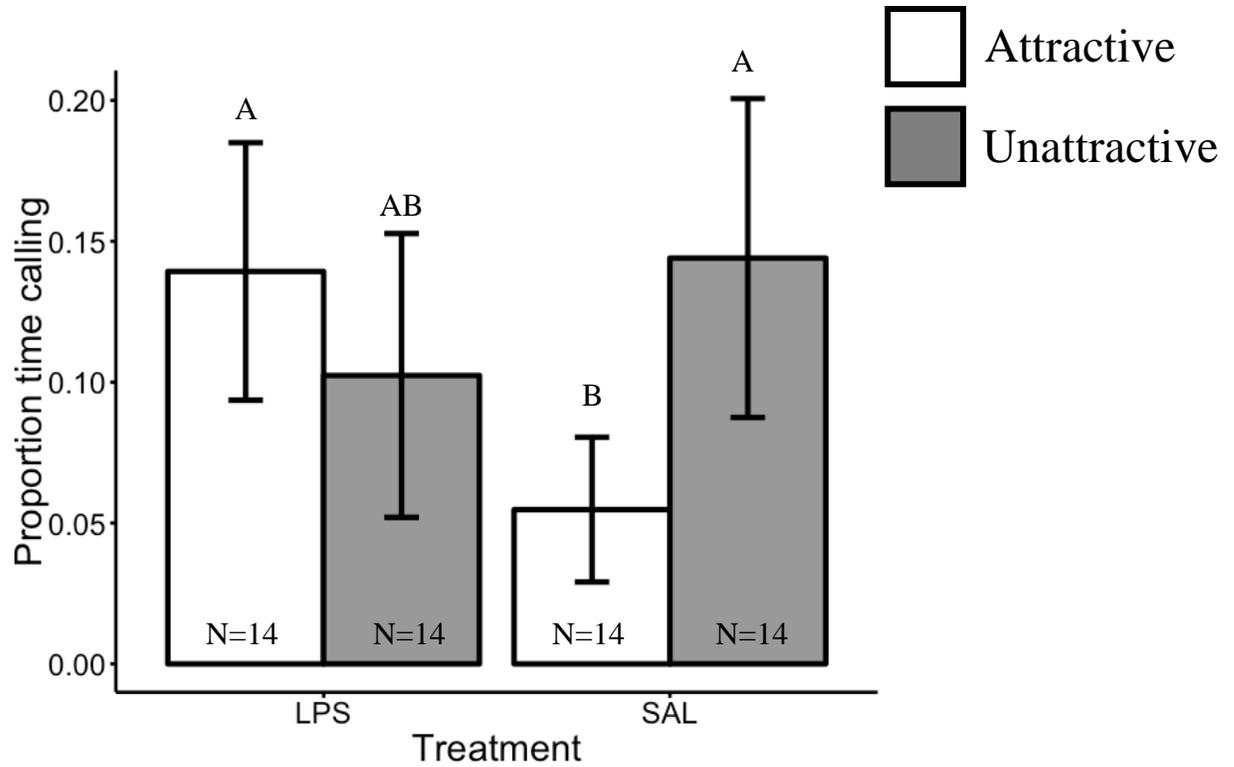
Figure 2: Proportion of time (± 1.0 S.E.) spent calling in attractive and unattractive male Texas field crickets *(Gryllus texensis)* injected with saline and lipopolysaccharide (LPS). Different letters above bars indicate significant differences (P < 0.05) (N=56, χ^2 = 6.009, *d.f.* = 1, *P* = 1.86e-09).
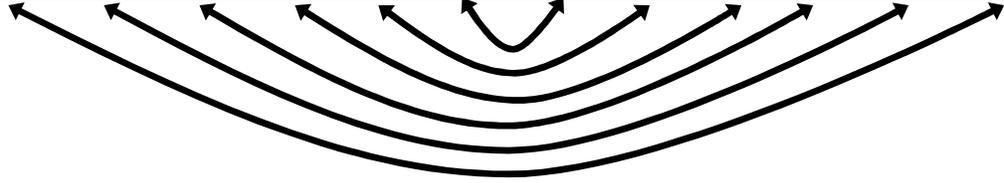
Figure 3: Mean (± 1.0 S.E.) proportion of time attractive and unattractive male Texas field crickets *(Gryllus texensis)* injected with saline (SAL) and lipopolysaccharide (LPS) spent hiding (N=56, $\chi^2 = 2.255$, *d.f.* = 1, *P* = 0.024).

APPENDIX

A.

Male	A	B	C	D	E	F	G	H	I	J	K	L
Female	O	P	Q	R	S	T	U	V	W	X	Y	Z
Rank 1	1	2	3	4	5	6	7	8	9	10	11	12

B.

Male	A	B	C	D	E	F	G	H	I	J	K	L
Female	Z	Y	X	W	V	U	T	S	R	Q	P	O
Rank 2	7	9	11	2	11	4	3	8	1	5	6	11

C.

Male	A	B	C	D	E	F	G	H	I	J	K	L
Female	U	T	O	X	R	V	P	W	Y	Z	Q	S
Rank 3	5	10	8	9	4	11	3	1	2	7	12	6

D.

Male	A	B	C	D	E	F	G	H	I	J	K	L
Female	O	P	U	R	X	Y	T	Q	V	S	W	Z
Rank 4	3	9.5	1	2	9.5	6	9.5	9.5	9.5	2	4	9.5

E.

| Male	A	B	C	D	E	F	G	H	I	J	K	L
Total Rank	16	30.5	23	17	29.5	27	22.5	26.5	21.5	24	33	38.5
Attractiveness	A	U	Discard	A	U	Discard	A	Discard	A	Discard	U	U

A1: ATTRACTIVENESS TRIAL SAMPLE FROM 12 MALES. Red numbers at the bottom of the cells are males that were not mounted. Day 1: A) Males paired randomly with a female. B) Fastest and slowest females switch males with each other. Day 2: C) Males were paired with a female they hadn't experienced. D) Fastest and slowest females switched with each other. E) Ranks were summed giving attractive, mid-ranking, and unattractive males. See methods for more detail.
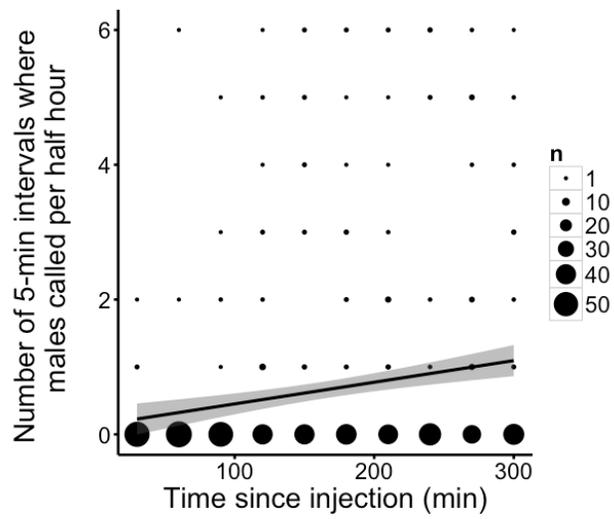
A2: CALLING. Number of times male Texas field crickets *(Gryllus texensis)* were observed calling in 5-minute intervals binned in 30 minutes. The shaded region depicts the 95% confidence interval.
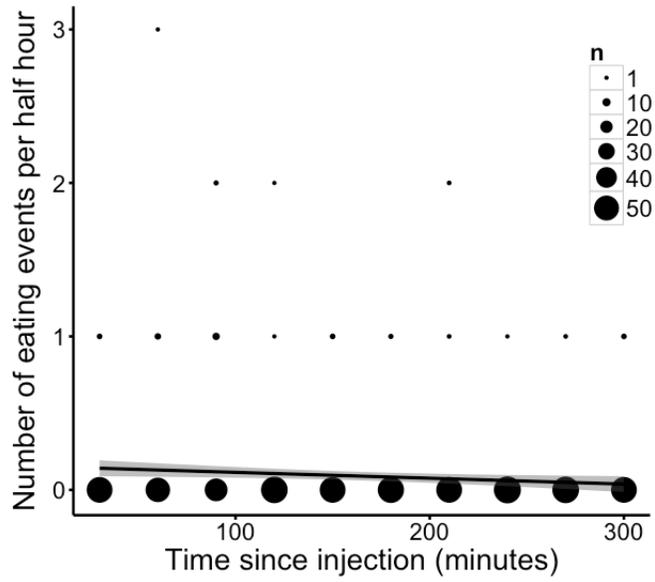
A3: FEEDING EVENTS. Number of times male Texas field crickets *(Gryllus texensis)* ate in 30 minutes increments. The shaded region depicts the 95% confidence interval (N=56, χ^2 = 12.071, *d.f.* = 1, *P* = 0.022).
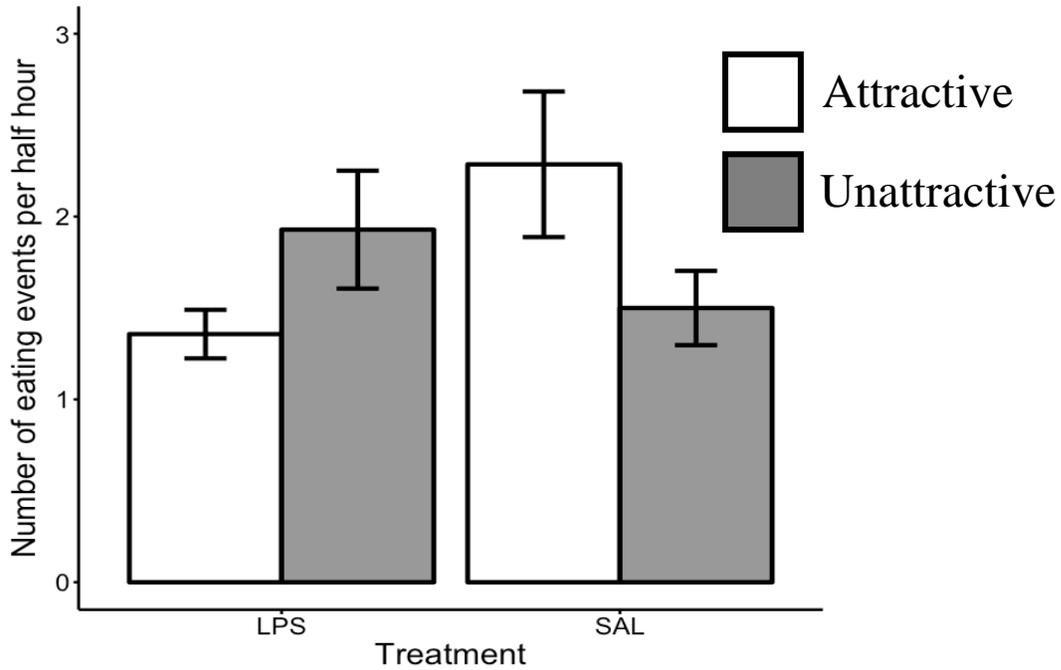
A4: MEAN FEEDING EVENTS PER HALF HOUR. Mean (± 1.0 S.E.) number of times attractive and unattractive male Texas field crickets *(Gryllus texensis)* injected with saline (SAL) and lipopolysaccharide (LPS) ate per 30 minutes. There was a significant interaction between attractiveness and injection treatment (N=56, χ^2 = 12.071, *d.f.* = 1, *P* = 0.022).
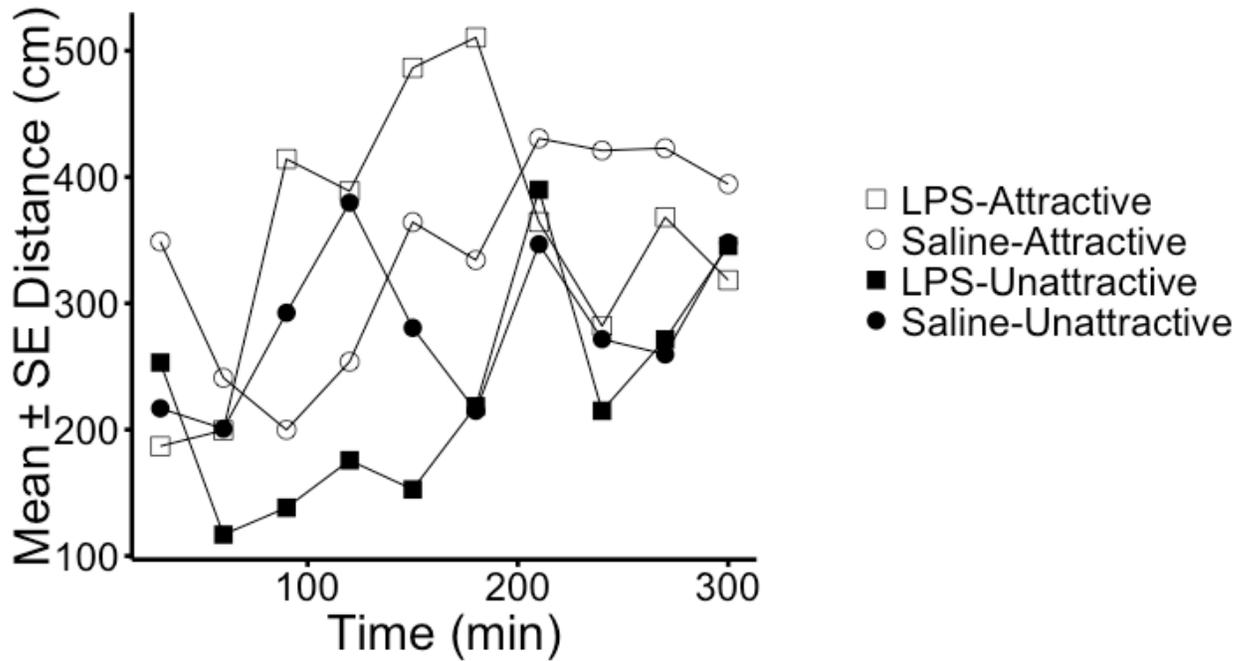
A5: MEAN DISTANCE TRAVELED. Mean distance traveled per half hour by attractive and unattractive male Texas field crickets *(Gryllus texensis)* injected with saline (SAL) and lipopolysaccharide (LPS). All groups increased the distance they traveled as the time since injection increased ($F_{1, 491.88} = 19.998$, $P = 9.636e-6$). There were no significant differences in distance travelled between attractiveness ($F_{1, 52.99} = 1.52$, $P = 0.223$) or injection treatment ($F_{1, 52.99} = 0.084$, $P = 0.774$).