

LIFE HISTORY RESPONSE TO INFECTION AND THE POTENTIAL FOR DISHONEST
SIGNALS IN THE GROUND CRICKET *ALLONEMOBIUS SOCIUS*

by

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ABSTRACT

In order to maximize fitness, individuals must partition their limited resources among competing physiological processes, creating negative statistical associations between processes known as “life-history trade-offs”. Evidence indicates that individuals tend to decrease their reproductive investment when confronted with a significant immunological challenge in order to increase investment in immune defense. This trade-off is often accompanied by a significant decrease in the sexual signal, which provides an honest signal of the male’s infection status to potential mates. However, if individual residual reproductive value is low, they may instead increase their reproductive investment to maximize reproductive success before the end of their life (a.k.a. terminal investment). Here, we investigate the potential for terminal investment in the ground cricket *Allonemobius socius* by inoculating males with varying dosages of an immune challenge. We predicted that both high dose and advanced male age would induce terminal investment. Furthermore, we predicted that terminally investing males would produce a dishonest signal by increasing their signaling effort. We found that upon infection We found that upon infection, young males and old males differentially alter their reproductive strategy. Young males exhibited the classic deceleration of reproductive effort. However, old males increased their calling song energetics and decreased their parental investment (nuptial gift size), suggesting that old males are dishonestly signaling their condition to the female.

I dedicate this thesis to the two people who have always encouraged me and believed in me, my mother, Bonnie, and my father, Steve. I would not be where I am today without their love and support.

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INTRODUCTION

Life history theory dictates that organisms must partition limited resources among competing physiological processes such as growth, somatic maintenance, and reproduction. Consequently, these costly processes must trade-off with one another in order to maximize individual fitness. For instance, when reproductively active individuals incur a significant pathogenic infection they are expected to increase investment in immune defense at the expense of reproductive investment (Allander and Bennett 1995, Adamo 1999, Uller et al. 2006). Interestingly, some evidence suggests that individuals may instead increase their reproductive effort when exposed to an immunological threat (Adamo 1999, Agnew et al. 2000, Schwanz 2008). Therefore, it appears that a host is confronted with the decision to either decelerate or accelerate its reproductive effort when confronted with a parasite. This decision is likely based on the host's residual reproductive value (RRV), which is defined as its future potential for reproduction. If RRV is high, one might expect the host to shift resources from reproductive effort to immune defense. However, if RRV is low, then a host may shift resources into reproductive effort from some other physiologically costly pathway.

The well-established negative correlation between reproductive effort and immune function found across numerous taxa (Schmid-Hempel 2003) suggests that the host will likely shift resources from immune defense, leading to an increased mortality rate. This latter strategy has been termed "terminal investment", in that the host's increase in reproductive effort allows the infection to proceed unchecked. Although the terminal investment hypothesis has generally been tested by examining the association between reproductive effort and age, one may expect

an individual to terminally invest whenever their RRV is low (e.g. when suffering from a detrimental infection). However, evidence for pathogen-mediated terminal investment is mixed (Adamo 1999, Agnew et al. 2000, Velando et al. 2006, Schwanz 2008).

One reason for the mixed evidence are the many factors which may confound the outcome of pathogen-mediated terminal investment studies, including an individual's age, sex, pathogen type, and pathogen load (Forbes 1993, Adamo 1999, Rolff 2002). Expected differences between the sexes in the propensity for terminal investment stems from the different fitness constraints placed on each sex. Male fitness is generally constrained by the number of successful (but infrequent) mating attempts, while female fitness is constrained by the rate of offspring production. Thus, males may terminally invest more readily because of a potentially greater fitness return compared with females. With regard to age, younger individuals may place a greater emphasis on immune defense considering the large amount of residual reproduction to be gained through recovery; making them less likely to invest terminally. Pathogen type may also influence investment strategy, as previous research in crickets has shown that infection with bacteria, but not parasitoids, influences reproductive output. It has been suggested that this pattern could be due to the fact that the cricket-parasitoid interaction is more evolutionarily recent than the cricket-bacteria interaction; so the response to parasitoids may not be fully evolved (Adamo 1999). Pathogen load should also be considered because if the infection dose is low, the individual may not be moribund and therefore may not terminally invest.

One aspect of reproductive effort that may be affected by an immune trade-off is the expression of "honest" sexual signals. Honest signals are costly to produce and therefore condition dependent, making them excellent targets for mate choice. Several evolutionary

models have suggested that these signals reflect the signaler's true ability to resist parasitic infection (Hamilton and Zuk 1982, Folstad and Karter 1992, Sadd et al. 2006). As would be expected under this hypothesis, pathogenic infection generally decreases signal expression (Ryder and Siva-Jothy 2000, Worden and Parker 2005, Fedorka and Mousseau 2007), allowing the chooser to avoid reproducing with an infected mate. However, terminally investing individuals may forgo immune investment and instead maintain signal investment. This would create a dishonest signal from the perspective of the chooser, who will unwittingly copulate with an infected mate (it should be noted that the signal is still honest with regard to the signaler in that a large signal results in a hastened mortality). Surprisingly, the potential for dishonest signal production among terminally investing individuals has rarely been investigated (Sadd et al. 2006).

Here we assess the potential for terminal investment in the southern ground cricket, *Allonemobius socius*. To this end, we manipulated the age, pathogen type and pathogen load of adult males and examined their resulting reproductive effort. A decrease in reproductive effort would suggest a classic life history trade-off strategy, while an increase in effort would suggest terminal investment. We predicted that individuals would be more likely to terminally invest if they were older, provided with a larger immune challenge or the immune challenge was bacterial in origin. Furthermore, we predicted that terminally investing males would attempt to mask their immune status from females by creating a dishonest signal in the form of increased signal energetics. In addition to testing the terminal investment hypothesis, this study provided the opportunity to examine the influence of male age on reproductive success. Previous work (Brooks and Kemp 2001) has suggested that females choose older males due to their proven

ability to survive throughout the breeding season. By doing so, females mating with older males would gain indirect genetic benefits in the form superior survival genes. We test this hypothesis by comparing the reproductive success of young and old males.

METHODS

System and Maintenance

The striped ground cricket, *Allonemobius socius*, is a small chirping cricket found throughout the southeastern United States. As with other cricket systems, males attract distant females with a conspicuous calling song. As a female approaches the male, he begins a courtship song and dance. Females likely use both the male calling song and courtship behavior as a measure of male quality (Wagner and Reiser 2000). If the female is receptive, she will mount the male and allow him to pass a spermatophore to her seminal receptacle. Once coupled, females chew on a specialized tibial spur on the male. This provides the female with a hemolymph-based nuptial gift until the pair separates (Fedorka and Mousseau 2002).

Experimental crickets originated from 200 wild caught individuals collected near Asheville, North Carolina in the summer of 2007. Individuals were kept in plastic cages and fed ground cat food and carrots *ad libitum*. Cages also contained dampened cheesecloth (oviposition material), water vials, and strips of brown paper and paper egg cartons for cover. All cages were kept in a Percival incubator (Boone, IA) at a constant 28°C and 15:9 (light:dark) light cycle. Prior to experimentation, all crickets were separated by sex as they approached adult eclosion and placed into sex-specific cages to ensure virginity. To assess the influence of pathogen type, virgin males were collected just after adult eclosion and randomly assigned to one of two immune treatments, including a simulated parasitoid attack (implant) treatment and a simulated bacterial infection (lipopolysaccharide) treatment (Figure 1). To assess the influence of male age

on the propensity to terminally invest, males from the LPS treatment were assigned to either a young or old mating treatment (9 ± 2 or 30 ± 2 days post eclosion, respectively). Due to resource constraints, only young males were used for the implant treatment.

Immune treatments

To assess the influence of a simulated bacterial infection, we injected both age groups with 1 μ l of lipopolysaccharides (LPS; extracted from gram-negative *Serratia marcescens*; Sigma-Aldrich, #L6136) diluted in insect Ringer's solution (Haine et al. 2007) directly into the hemocoel. To this end, individuals were cold anaesthetized on ice for five minutes, and LPS was dispensed using a microsyringe (Hamilton) inserted between the 2nd and 3rd abdominal sternites. Considering that we wished to elicit both the general life history strategy (assumed to be elicited through a small infection) and the terminal investment strategy (assumed to be elicited through a large infection), we randomly assigned males to one of six LPS dosage treatments (0.01 μ g, 0.1 μ g, 1.0 μ g, 10 μ g, 50 μ g, and a 0.0 μ g control; Figure 1). After injection, males were placed into individual 5cm petri dishes containing a carrot slice and water-soaked cotton for 24 hours prior to having their calling song and mating parameters assayed.

To assess the influence of a simulated parasitoid attack, we cold anaesthetized males for five minutes on ice. After major motion had ceased, we created a hole between the 2nd and 3rd abdominal sternite using a sterile 28 gauge needle. Into this hole we inserted a 2mm long piece on nylon monofilament. Again, in an attempt to elicit both immune response strategies, we randomly assigned males to one of six implant treatments based on implant diameter (0.009mm,

0.11mm, 0.12mm, 0.13mm, 0.15mm and a control; the control had no implant inserted after abdominal perforation with the needle; Figure 1). As with the LPS treatment, after treatment males were individually placed into petri dishes for 24 hours prior to being placed with the female.

Mating and Calling Song Assay

In order to determine the reproductive strategy (trade-off or terminal investment) exhibited by immune challenged males, males were placed into a mating arena (10 cm petri dish) with a virgin female and allowed to interact for one hour. All assays occurred under a red light in a temperature controlled room maintained at 25°C. Measures of reproductive effort included the production of a calling song (0/1), mating success (0/1), latency to mate (time from entry into the mating arena to mating, and mating duration (time from the onset to the end of mating). In addition, males had their calling song (the sexual signal) recorded using a sound level meter (Realistic Sound Level Meter #33-2050) attached to a computer. Several song components were estimated including interchirp interval (ICI), interpulse interval (IPI), chirp duration (CD), chirps per minute (CPM), and pulses per chirp (PPC; Figure 2). For each song characteristic, three estimates were taken per male and then averaged to obtain the final value. Song components were estimated using Sound Ruler acoustic analysis software (<http://soundruler.sourceforge.net/>). After being separated from the female, male femur length was recorded.

Additionally, after interacting with the female, males treated with implants were again cold anaesthetized and the implant was removed from their abdominal cavity. Implants were stored at -80°C until they were ready to be measured for melanization.

Statistical analysis

All statistical analyses were conducted using JMP version 8 (SAS Institute, Cary, North Carolina). Nominal logistic regressions were used to investigate the association between song parameters (IPI, ICI, CD, PPC, and CPM) and mating success. Each parameter was tested independently without covariates. Considering that these song components represent different measures of song energetics, they are inter-correlated. Therefore, we performed a principle component analysis to create several uncorrelated song energetic variables, which were also tested against mating success. Multivariate ANOVAs were used to investigate the influences of treatment and age on all calling song parameters (including principle components 1 and 2). A Student's t-test was used for paired comparisons among treatments. Non-parametric cubic splines were created to visualize the relationship between mating success and calling song parameters (Schluter 1988). To obtain a measurement of confidence around the estimated function, each spline was bootstrapped 500 times. With respect to other mating parameters, nominal logistic regressions were used to investigate the association between song production (sang, silent) and mating success (mated, unmated) with age and treatment. Multivariate ANOVAs were used to investigate the influence of treatment and age on latency to mate and mating duration. To test for paired comparisons among treatments, a Student's t-test was used.

For these analyses, body size was used as a covariate. Outliers were removed using Grubbs' test for outliers. For all models, non-significant interaction terms were discarded and the models reanalyzed.

RESULTS

Calling song parameters

Male calling song was significantly associated with mating success. Interchirp interval (ICI), pulses per chirp (PPC) and chirp duration (CD) all exhibited a negative relationship with mating success, while chirps per minute (CPM) exhibited a marginally significant positive relationship (Table 1). Although these opposing patterns seem contradictory, CPM was negatively correlated with ICI, PPC and CD ($r = -0.59, -0.34, -0.31$, respectively; all $P > 0.0001$). This pattern was further supported by the negative relationship between mating success and the first principle component (PC1), which we interpret as an overall descriptor of song energetics. Thus, males with the most energetic songs were the most successful at acquiring mates.

Both immune activation and age had a significant influence on various male calling song components. Males treated with lipopolysaccharides (LPS) showed significant change in CPM, with males of both ages exhibiting a similar complex combination of deceleration and acceleration in song energetics with increasing LPS dose (Table 2; Figure 3a). Furthermore, we found that both age and treatment interacted to significantly influence ICI and PC1 (Figure 3b), with a marginal influence on PPC (Table 2). In all cases, younger males tended to exhibit the stereotypical reduction in reproductive effort upon infection by reducing their song energetics. In contrast, older males exhibited acceleration in reproductive effort, producing a more energetic calling song as LPS dose increased. The CPM pattern appears somewhat inconsistent with the ICI and PPC patterns. Considering that PC1 integrates variation in all song components, we feel

that the PC1 pattern most likely represents the true relationship between LPS dose and song energetics (Figure 4). This is a fascinating observation that suggests that old and young males exhibit drastically different life history strategies in response to a perceived parasitic infection.

For males treated with implants, we did not find any relationship between calling song characteristics and treatment. Furthermore, we did not find a relationship between the degree of melanization on the implant and treatment, age, or calling song parameters.

Mating parameters

With respect to males treated with lipopolysaccharides (LPS), treatment and age had no influence on the probability of producing a calling song (76% of all males called) or on the latency to mate (~36 minutes after first contact with the female; Table 3). However, age had a significant influence on male mating success, with younger males acquiring mates more often than older males (Figure 7a). Age and treatment both had an influence on mating duration, which provides an estimate of how much nuptial gift (i.e. hemolymph) is transferred to the female during mating. Older males exhibited a significant decline in the duration of mating as LPS dose increased (Figure 6). However, younger males exhibited no overall decrease in mating duration. Interestingly, young males exhibited a spike in mating duration when inoculated with the smallest dose of LPS, suggesting that a minor perceived infection caused them to overinvest in mating. With regard to older males, it appears that as the perceived threat of infection increases, they increase their song energetics and decrease their nuptial gift size. These patterns suggest that males dishonestly signal their infection status and paternal investment ability.

For males treated with implants, we did not find any relationship between mating parameters or treatment. Additionally, we did not find a relationship between the degree of melanization on the implant and treatment, age, or mating parameters.

DISCUSSION

In this study, we assessed the influence of pathogen load, pathogen type, and age on the sexual signal and reproductive effort of male ground crickets. We first predicted that song energetics would be associated with male mating success. Additionally, we predicted that if a male's residual reproductive value was high, they would decelerate reproductive effort and signal; a behavior that is stereotypical of a life history trade-off. However, as residual reproductive value decreases, we predicted that males would alter their strategy and accelerate reproductive effort; a behavior that is stereotypical of terminal investment. Importantly, we hypothesized that terminally investing males would produce a dishonest signal in an attempt to mask their infection status. We also predicted that male age would influence the above behaviors, with older males being more prone to accelerating their reproductive effort upon infection. We found support for each of these predictions.

With regard to our first prediction, we found that male calling energetics were positively associated with mating success (Table 1, Figure 5). Specifically, song interchirp interval (ICI), pulses per chirp (PPC), chirps per minute (CPM) and chirp duration (CD) were all negatively associated with a male's ability to acquire a mate. These data are in support of previous work which shows that *Allonemobius* females prefer more energetic song characteristics (Fedorka and Mousseau 2007, Olvido et al. 2010). In support of our second prediction, we show that young males decelerated their calling song energetics upon infection (Figure 4). Young males in our experiment likely possessed ample residual reproductive value prior to infection, considering that adult *A. socius* can live upwards of 2 months. Thus, their high RRV likely leads to a deceleration rather than acceleration of reproductive effort. This is not surprising as trade-offs between

reproductive effort, such as the sexual signal, and immune function are well documented (Lawniczak et al. 2007). For instance, (Jacot et al. 2004) found that daily calling rate of the field cricket, *Gryllus campestris* decreased upon immune activation. Additionally, Ryder and Siva-Jothy (2000) found that female *A. domesticus* preferred males with more syllables (pulses) per chirp and that this trait correlated with variation in immune function.

Interestingly, old males did not exhibit the same response in song energetics as young males. Although older males have a lower RRV compared with younger males, we expected them to decrease their sexual signal at low doses of pathogen, but accelerate their signal at high doses (i.e. terminally invest). This expectation assumed that RRV was high at a low LPS dose, but steadily decreased with increasing dosage. We found that even at low doses of pathogen, old males accelerated their reproductive effort, producing a more energetic song. These data show that while young males took the classic approach of decelerating reproductive effort (calling song energetics) upon infection, older males increased their calling song energetics upon infection.

Although surprising, this pattern is consistent with theory, given the fact that older males already have a lower residual reproductive value (RRV) than young males and therefore may be more prone to accelerate their reproductive effort, even if it leads to hastened mortality. This could be perceived as a dishonest signal from the female's perspective, as the male is not accurately reflecting his infection status. This contradicts previous work which suggests that males age and residual reproductive value decreases, signal becomes more reliable (Proulx et al. 2002). Additionally, (Hall et al. 2009) found that male wrens called more during their last year of life, suggesting that as residual reproduction decreases, males invest more in their sexual signal.

In addition to calling song, we also examined several other mating parameters that reflect male reproductive effort, including latency to mate and mating duration. We found that treatment had a significant influence on mating duration, but not latency to mate. However, we did not see the patterns that we originally expected. We found that for both young and old males in the control group, there was no difference in mating duration, which provides an estimate of hemolymph transfer to the female (i.e. nuptial gift size). However, when LPS was administered, their mating durations differed. Young males showed no overall change in the duration of mating. However, they exhibited a much longer mating duration when treated with the smallest dose of LPS. This is an intriguing pattern; however, we are not certain for the reason of this overinvestment indicating the need for further studies. As for older males, as the LPS dose increased, their mating duration decreased (Figure 6). This infers that as the perceived threat of infection increased, their song energetics increased yet their nuptial gift size decreased. This, again, suggests that older males dishonestly signal their infection status and paternal investment ability.

Previous work suggests that older males make for better mates because they have survived for a longer period, indicating their “good genes” (Brooks and Kemp 2001). To investigate this, we examined the influence of age (independent of treatment) on mating parameters. Although both young and old males call at the same rate, we showed that young males were more likely to mate than older males (Figure 7a). Additionally, we found that once they were successful at mating, younger males mated for a longer period of time than older males, thus giving more parental resources (larger nuptial gift) to the female (Figure 7b). This suggests that old males are less attractive mates than young males. These findings contradict the

hypothesis that females prefer old males. Additionally, they contradict previous studies in birds and crickets that have shown that older males have a greater parental investment (parental care, nuptial gift size) than younger males (Forslund and Part 1995, Velando et al. 2006, Lehmann and Lehmann 2009).

This study suggests that males can differentially modify their life history response depending on the severity of infection. However, the response to infection could be the result of two things. First, males may have modified their response based on the shift in energy allocation as their immune systems were being activated (indirect effect of pathogenic infection). Secondly, this response may be due simply to the damaging effects of LPS, which is an endotoxin (direct effect of pathogenic infection). Although LPS is non-replicating and only activates part of the immune cascade that whole bacteria elicit, previous work has shown similar trends in reproductive behavior when organisms are treated with bacteria and with LPS (Adamo 1999). The pattern of accelerated reproduction we documented is often referred to in the literature as terminal investment. However, since we did not track mortality, this pattern cannot be deemed as such, as the term terminal suggests mortality. Furthermore, this is the first study to document the fact that young males and old males differentially alter their reproductive effort upon immune activation, with old males producing a dishonest signal.

While we did not see a change in reproductive effort when we treated males with implants, this was not unexpected as previous work in crickets has yielded similar results. It has been suggested that this lack of response may be due to the fact that the cricket-parasitoid interaction may be more recent than the cricket-bacteria interaction and thus, not as sensitive

(Adamo 1999). Lastly, a different pattern may have been seen if we had also treated old males with implants.

Since this study was completed only in males, future studies should be carried out to determine whether or not we would see the same response in reproductive effort in females as we see in males. Additionally, *A. socius* is a bivoltine species, with a summer stage and a winter stage. As individuals in the winter stage are under a stronger pressure to reproduce before the season ends, they have a lower residual reproduce value than individuals in summer conditions. Thus, differences in reproductive effort may be expected between the two stages. In fact, (Olvido and Mousseau 1995) found that calling song frequency of adult *Allonemobius fasciatus* was influenced by environmental temperature. This study was completed using individuals in the summer conditions, so the response in winter stage individuals should be further investigated. Lastly, since we did not investigate changes in immune status, further work is needed to elucidate the physiological mechanism that underlies this pattern.

APPENDIX: FIGURES AND TABLES

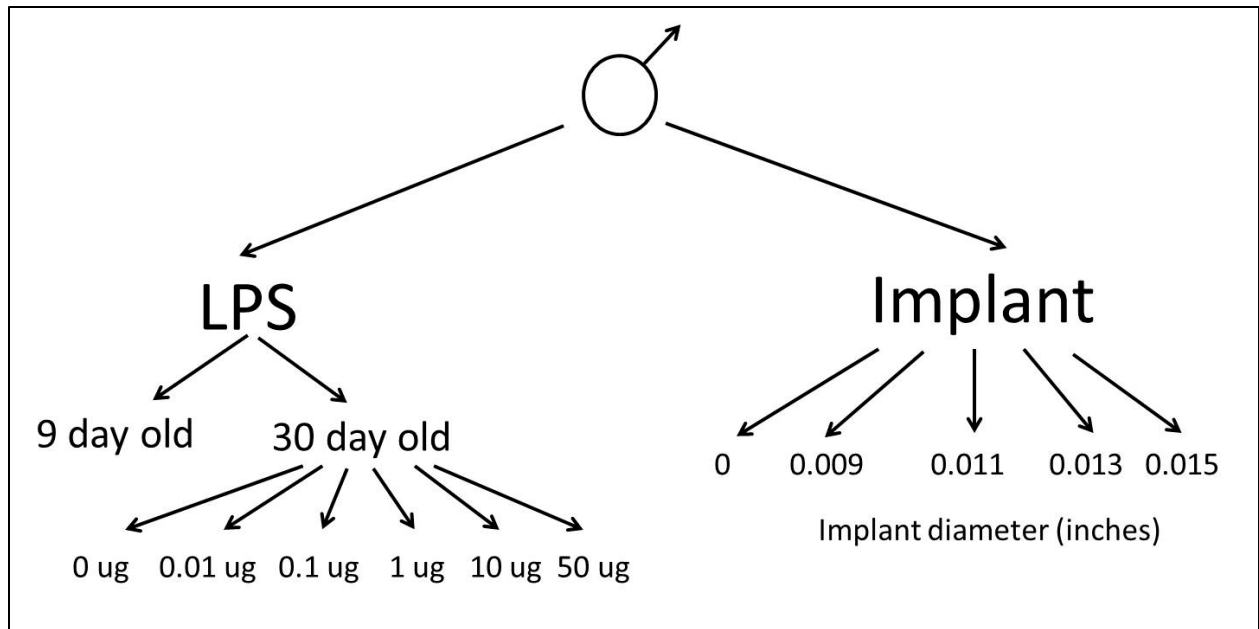


Figure 1: Experimental design. Males were randomly assigned to either an LPS or implant treatment group. From there, LPS males were assigned to either an old age group or a young age group. Males were also equally placed into treatment groups.

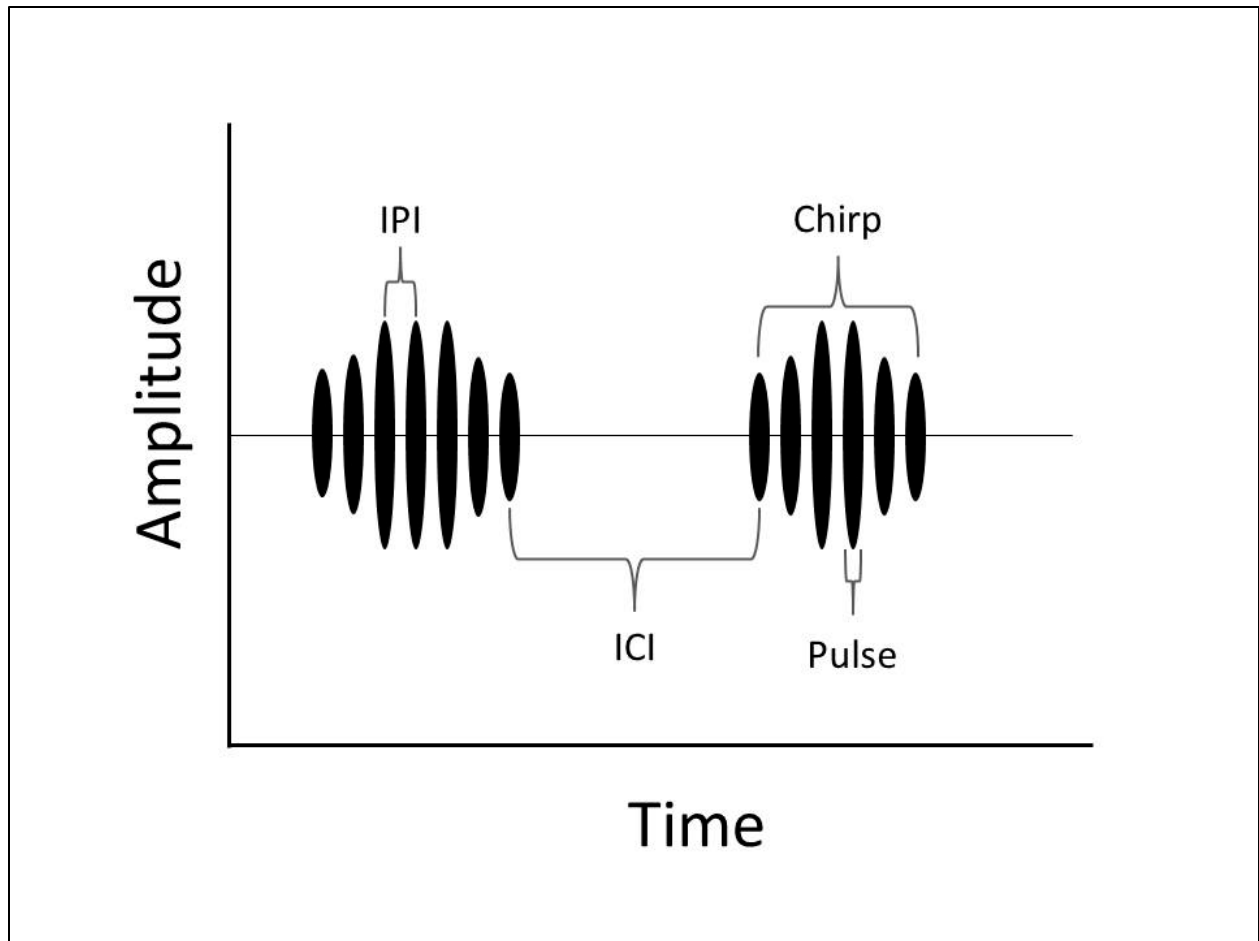


Figure 2: Male calling song oscillogram. Several characters of calling song were estimated in this study including interpulse interval (IPI), interchirp interval (ICI), chirp duration (CD), pulses per chirp (PPC) and chirps per minute (CPM).

Table 1: The association between mating success and calling song parameters. ICI, PPC, CD, and PC1 were negatively associated with mating success.

Independent	Direction	χ^2	p-value
IPI	0	0.004	0.9488
ICI	-	8.23	0.0041
PPC	-	5.47	0.0193
CD	-	4.86	0.0274
CPM	+	3.08	0.0794
PC1 (65%)	-	7.57	0.0059
PC2 (23%)	0	0.19	0.6614

Table 2: The association between calling song parameters, age, and treatment. The interaction between age and treatment was significant for ICI and PC1.

Dependent	Independent	df	Statistic	p value
IPI	Age	1, 280	F = 1.54	0.2163
	Treatment	5, 276	F = 1.21	0.3061
	Femur	1, 280	F = 4.99	0.0262
ICI	Age	1, 280	F = 0.84	0.36
	Treatment	5, 276	F = 0.93	0.4619
	Femur	1, 280	F = 2.51	0.1141
	Age*Treat	5,276	F = 2.71	0.0206
PPC	Age	1, 280	F = 0.20	0.6524
	Treatment	5, 276	F = 0.92	0.4660
	Femur	1, 280	F = 0.12	0.7310
	Age*Treat	5,276	F = 1.94	0.0880
CD	Age	1, 280	F = 0.06	0.8026
	Treatment	5, 276	F = 0.79	0.5552
	Femur	1, 280	F = 0.13	0.7179
CPM	Age	1, 280	F = 0.60	0.4393
	Treatment	5, 276	F = 2.69	0.0214
	Femur	1,280	F = 5.17	0.0238
PC1	Age	1, 279	F = 0.07	0.7889
	Treatment	5,275	F = 1.42	0.2156
	Femur	1, 279	F = 2.00	0.1587
	Age*Treat	5,275	F = 2.44	0.0351
PC2	Age	1, 279	F = 0.08	0.7799
	Treatment	5, 275	F = 1.72	0.1309
	Femur	1, 279	F = 3.35	0.0684

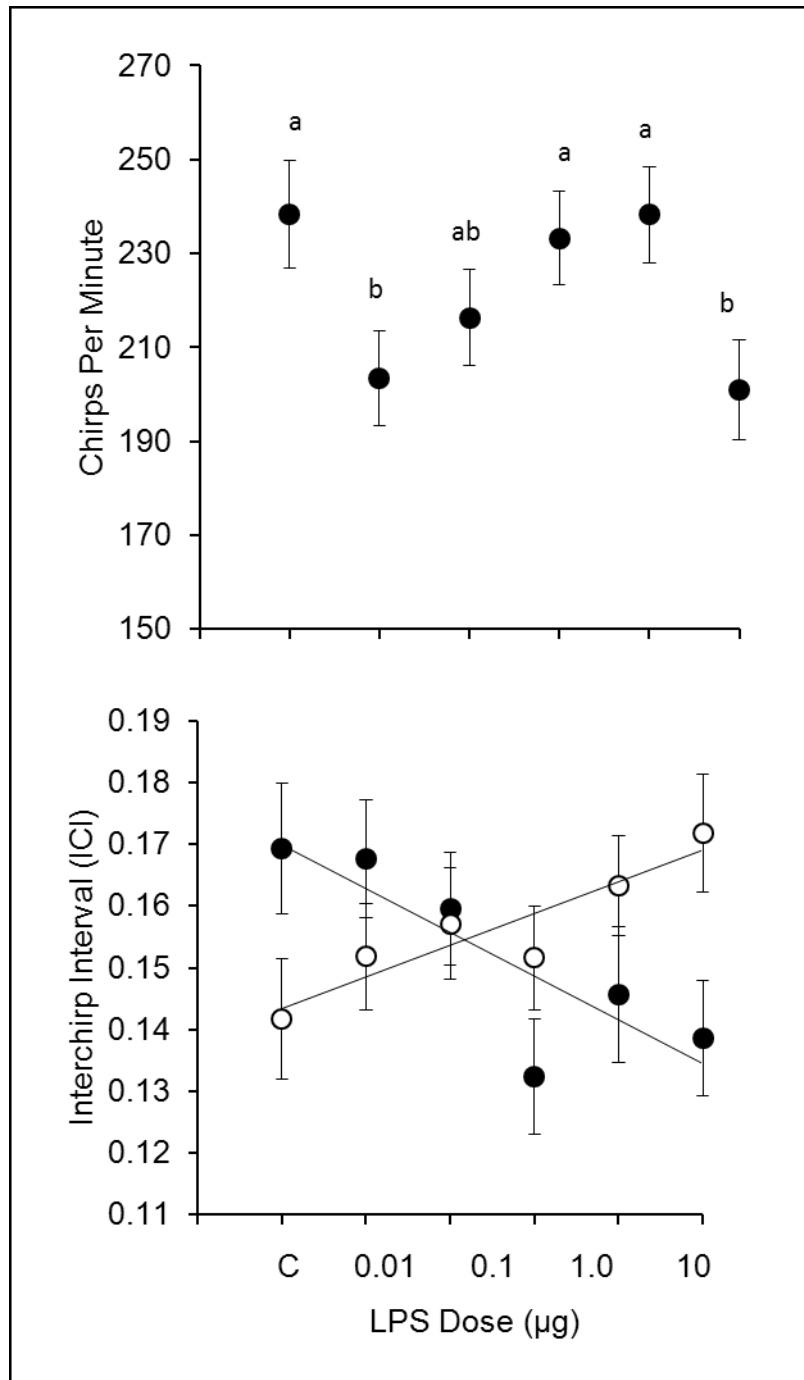


Figure 3: The association between ICI, CPM, age, and treatment. As dose increased, young males decreased their song energetics while old males increased their song energetics.

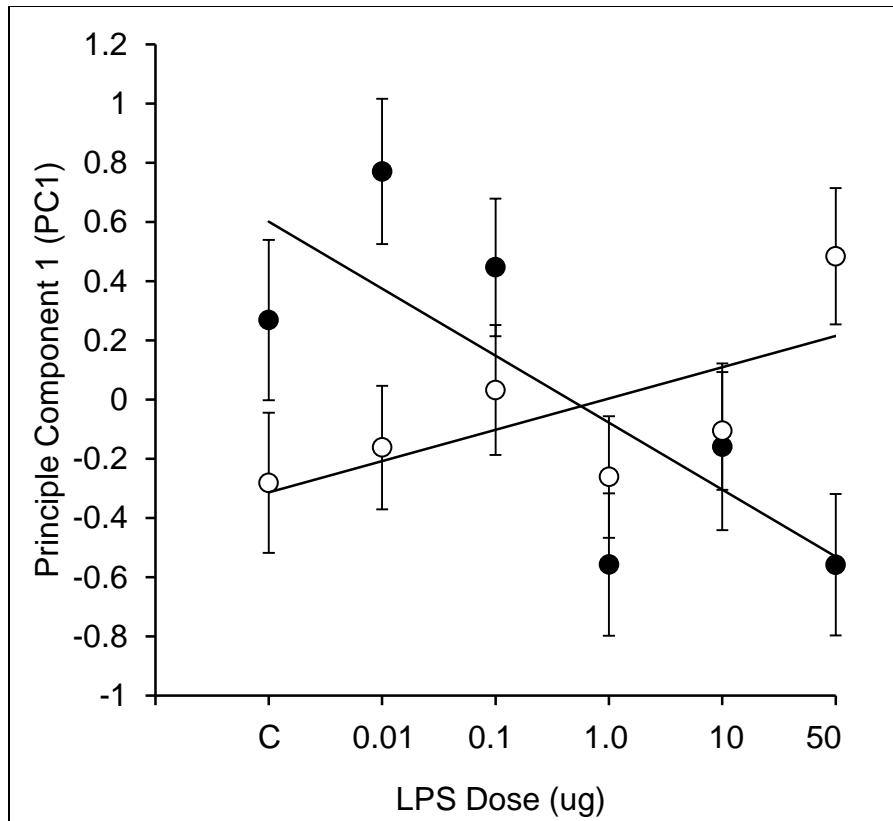


Figure 4: The association between PC1, treatment, and age. Old males increased their calling song energetics upon infection while young males decreased their song energetics upon infection.

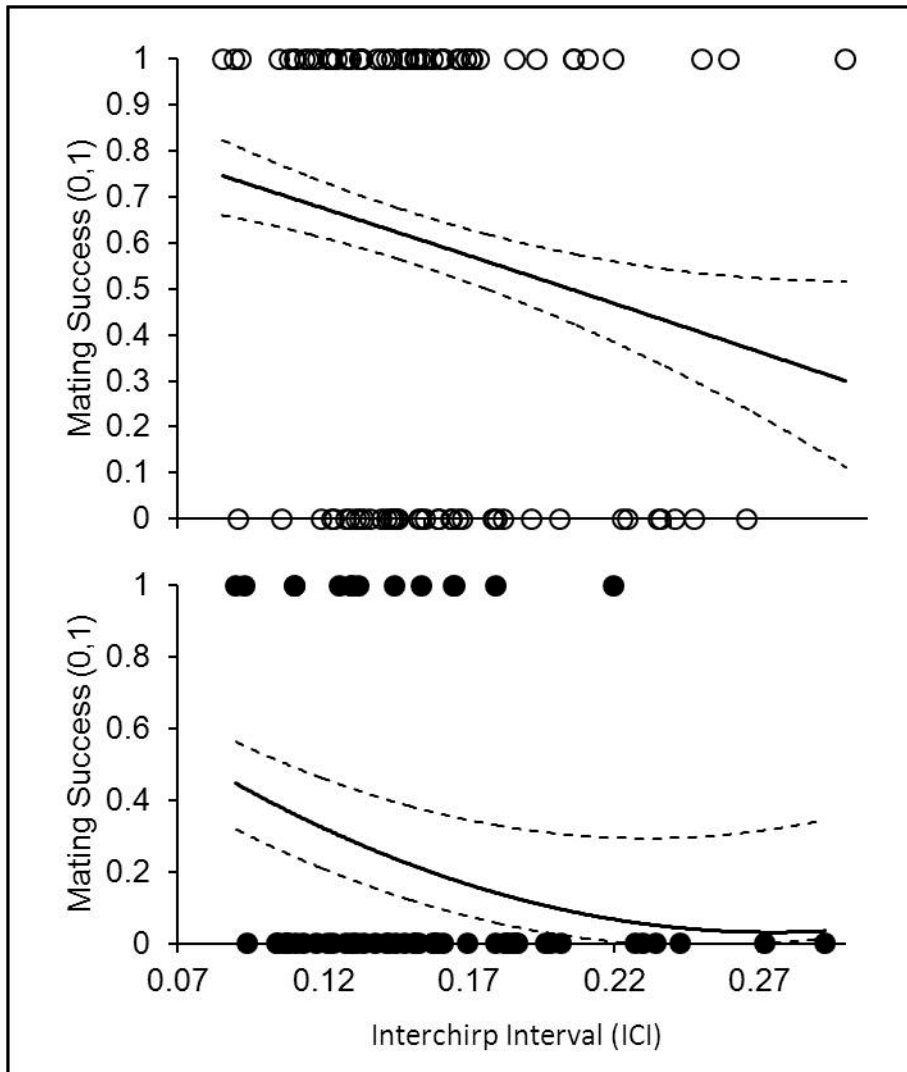


Figure 5: The association between mating success and interchirp interval. Males with more energetic songs (lower ICI), had a higher probability of mating success. This trend was seen in both young males (open circles, top) and old males (closed circles, bottom).

Table 3: The influence of age and treatment on mating parameters.

Dependent	Independent	df	Statistic	p value
Song Production (0, 1)	Age	1, 360	$\chi^2 = 0.25$	0.6145
	Treatment	5, 356	$\chi^2 = 4.38$	0.4956
	femur	1, 360	$\chi^2 = 0.27$	0.604
Latency	Age	1, 128	$F = 0.01$	0.9097
	Treatment	5, 124	$F = 0.30$	0.9118
	femur	1, 128	$F = 0.01$	0.0094
Mating success (0, 1)	Age	1,360	$\chi^2 = 36.09$	0.0001
	Treatment	5, 356	$\chi^2 = 8.01$	0.1559
	femur	1, 360	$\chi^2 = 0.26$	0.6132
Mating duration	Age	1,128	$F = 11.87$	0.0008
	Treatment	5, 124	$F = 3.14$	0.0106
	femur	1, 128	$F < 0.01$	0.9903

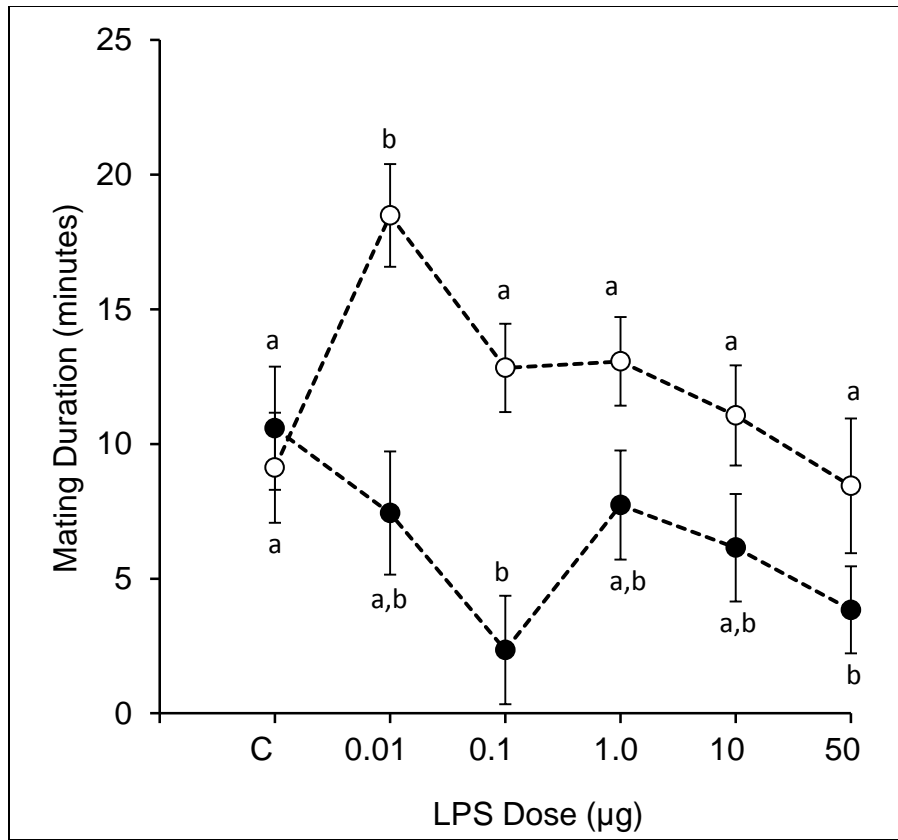


Figure 6: The association between treatment, mating duration, and age. Old males exhibited an overall decrease in mating duration as treatment increased, but young males did not exhibit a change.

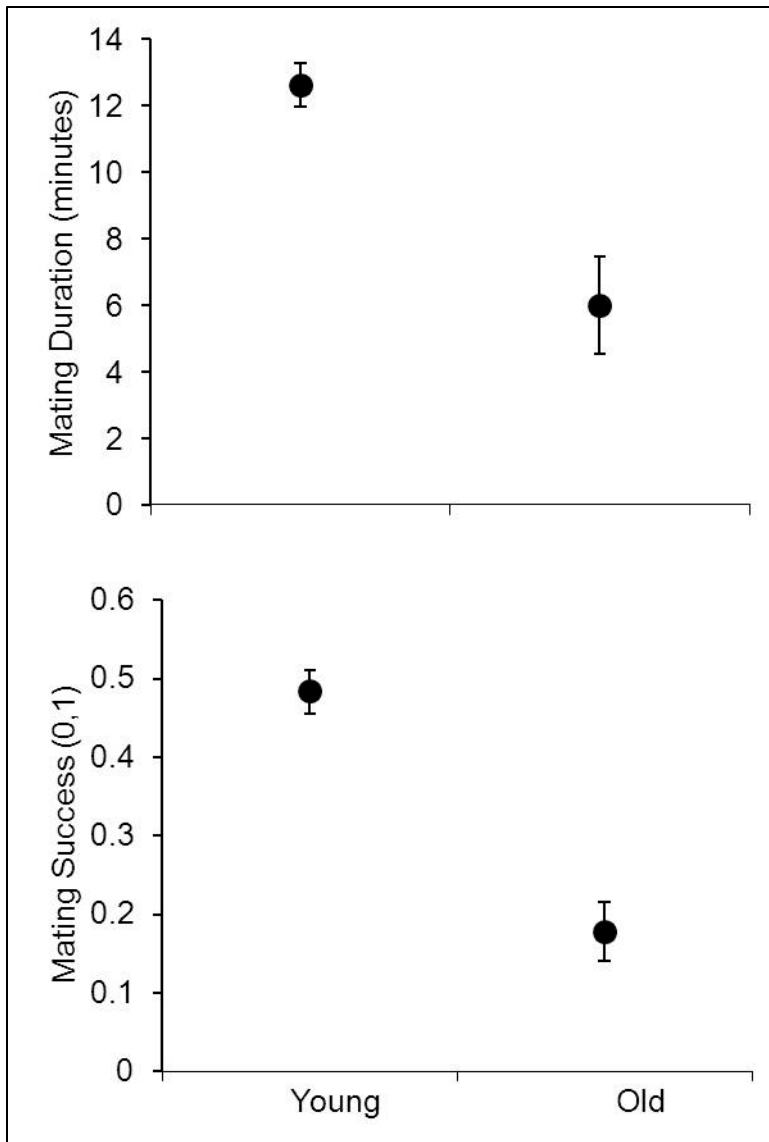


Figure 7: The influence of age on mating success and mating duration. Young males had a higher probability of mating and also had a longer mating duration upon mating.

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