Title

Consequences of the Evolutionary Loss of a Sexual Signal in the Field Cricket Teleogryllus oceanicus

Author

Gray, Brian

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Consequences of the Evolutionary Loss of a Sexual Signal in the Field Cricket *Teleogryllus oceanicus*

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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in

Evolution, Ecology, and Organismal Biology

by

Brian M. Gray

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Dissertation Committee:
  Dr. Marlene Zuk, Chairperson
  Dr. David Reznick
  Dr. Joel Sachs

The Dissertation of Brian M. Gray is approved:

Committee Chairperson

University of California, Riverside

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ABSTRACT OF THE DISSERTATION

Consequences of the Evolutionary Loss of a Sexual Signal in the Field Cricket *Teleogryllus oceanicus*

by

Brian M. Gray

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
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Evolutionary events have potentially dramatic implications for organisms. I took advantage of an evolutionary event, the loss of a sexual signal in the field cricket *Teleogryllus oceanicus*, to elucidate the potential consequences of such a change. In some Hawaiian populations, males possess a wing mutation, *flatwing*, that precludes song production, rendering them obligately silent. Signal loss has the effect of altering the acoustic landscape experienced by all crickets, representing a change in the social environment. Previous studies indicate that the lack of acoustic input alters several phenotypic traits, perhaps by changing perceived population density. I investigated the role of the acoustic environment in mediating male reproductive investment and aggressive behavior. Males reared in song-dense environments, representing populations with many rivals, invested more in the ejaculate but were less aggressive than males from silent environments.

In addition, because flatwing males lack the ability to produce song, they face difficulties in attracting mates, since females locate males by responding to calling song. In some other species with multimodal communication, individuals may compensate for the temporary loss of a signal modality by switching to or increasing the use of an alternative, pre-existing modality. Communication in my study species involves acoustic and chemical cues, so I predicted that males lacking the ability to produce song would compensate by producing more attractive chemical cues. A female-response assay showed that females responded to chemical cues from both normal-winged and flatwing males, but did not exhibit a preference for either, indicating that flatwing males do not compensate for a lack of song with chemical cues.

Further, on the Hawaiian islands, calling males are subject to lethal parasitism from an acoustically-orienting North American parasitoid fly, *Ormia ochracea,* and males in parasitized populations do not live as long as males from unparasitized populations. Given that flatwing males are unable to produce song and are thus protected from parasitism from *O. ochracea*, I predicted that flatwing males would have a greater average age than their normal-winged counterparts in the same population. Contrary to my predictions, flatwing males were not older than normal-winged males.

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DISSERTATION INTRODUCTION

Sexual signals are ubiquitous and encompass a range of traits, from bright coloration to weaponry and extravagant behavioral displays (Andersson 1994). While sexual signals may serve to attract potential mates and provide information about the quality of the signaller, sexual signals can also be used in a variety of other contexts, such as in transmitting social information to both conspecific and heterospecific individuals (reviewed in Kasumovic and Brooks 2011). This social information may provide individuals with clues about local mate competition or population density, and the effects of social cues on traits have been particularly well-studied in the areas of reproductive investment (Kasumovic and Brooks 2011). For example, males of a number of species increase reproductive investment in response to increased risk of sperm competition (delbarco-Trillo 2011, Kelly and Jennions 2011). Social cues appear to modulate perceived sperm competition risk in a number of taxa (though see Schaus and Sakaluk 2001, Ramm and Stockley 2009), including insects (Larsdotter Mellström and Wiklund 2009, Allen et al 2011), mammals (delBarco-Trillo and Ferkin 2004, Pound and Gage 2004), and humans (Kilgallon and Simmons 2005). Social cues can also influence a number of other, non-reproductive traits (reviewed in Kasumovic and Brooks 2011; see below).

However, the expression of sexual signals can also incur a fitness cost to individuals. That is, many traits used in mate advertisement or mate acquisition come at a cost to their bearers, in terms of fitness, energetic expenditure, or both. Thus, sexual signals often represent a compromise between the conflicting forces of natural and sexual selection (Darwin 1874, Andersson 1994). For example, many advertisement calls may be utilized by predators or parasitoids to locate suitable prey or hosts (reviewed in Zuk and Kolluru 1998). In perhaps one of the best known examples of natural selection counteracting sexual selection, female guppies prefer male guppies that are more brightly colored (e.g. carotenoid and structural coloration) (Endler 1983, Kodric-Brown 1985). However, males with brighter coloration are also more visible to predators, and thus, more frequently attacked (Godin and McDonough 2005). Sexual signals can also make it more difficult to escape from predators (Andersson 1994). In the sex-role reversed long-tailed dance fly, females inflate abdominal sacs to appear larger to males, but these sacs make females more likely to become ensnared in webs of predatory spiders (Gwynne et al 2007).

Differences among populations in the relative strength of natural and sexual selection may lead to variation in the expression of sexual signals among populations (Bertram et al 2004, Bentsen et al 2006). For example, in the aforementioned guppies, the extent of male coloration decreases with increasing predation pressure across populations (Endler 1983). In extreme cases, selection can even lead to the evolutionary loss of a trait. Consequently, we might expect the loss of a sexual signal that also provides social information to conspecifics to have particularly widespread ramifications. While sexual signal loss appears to be relatively common (Wiens 2001), such loss and any potential and concomitant consequences are rarely observed on a contemporary timescale (Svensson and Gosden 2007).

For my dissertation, I took advantage of a unique situation in which a sexual signal has been lost on a contemporary timescale. The field cricket *Teleogryllus oceanicus* has a wide distribution (Otte and Alexander 1983, Otte 1994), and was introduced to Hawaii by 1877 (Kevan 1990). Throughout the range, males sing to attract females, but in Hawaii, singing also attracts an acoustically-orienting parasitoid fly, *Ormia ochracea* (Zuk et al 1993, 1995, 2006). *O. ochracea* deposits live larvae on and around calling males, and the larvae burrow into the male where they consume tissue before emerging 7-10 days later, killing the host in the process (Adamo et al 1995).

Consequently, calling in Hawaii is a risky but necessary endeavor for males, and so represents a conflict between natural and sexual selection. Males must call to attract mates (sexual selection) but doing so increases risk of lethal parasitism (natural selection). These opposing selective forces have led to a number of shifts in cricket physiology, morphology, and behavior (Zuk et al 1993, Rotenberry et al 1996, Lewkiewicz and Zuk 2003). The most dramatic change is the evolutionary loss of a sexual signal, male song (Zuk et al 2006), the result of a single-locus, sex-linked mutation (Tinghitella 2008) that leaves bearers, hereafter *flatwings*, lacking the wing structures necessary to produce sound. The loss of this signal seems to have been accommodated by a relaxation of female preferences for acoustic signals in island populations of *T. oceanicus* (Bailey et al 2008, Tinghitella and Zuk 2009). This loss also represents one of the fastest cases of microevolution documented in the wild, having occurred in fewer than 20 generations (Zuk et al 2006). The consequences of this are potentially profound, and the evolutionary loss of a sexual signal consequently generates a number of intriguing situations to test outstanding questions in evolutionary biology.

Situation 1) Alteration of acoustic experience

The loss of a sexual signal that serves as a social cue rapidly altered the acoustic landscape experienced by conspecifics. In *T. oceanicus* and other crickets, acoustic cues are of critical importance and seem to play a key role in estimating local population density. The alteration of acoustic experience may have dramatic consequences on cricket behavior and physiology. In *T. commodus*, males exposed to song-dense environments become larger at maturity and called less than males reared in environments with less acoustic input (Kasumovic et al 2011). In *G. integer*, males reared in acoustically-dense environments are less aggressive than males reared in silent environments (DiRienzo et al 2012). In my study species *T. oceanicus*, males exposed to song-dense environments as juveniles attain greater body mass, invest more in reproductive tissue mass and are less likely to act as satellites (Bailey et al 2010). Males reared in acoustically-rich environments also produce less attractive cuticular hydrocarbons (Thomas et al 2011) and have greater immunity (Bailey et al 2011), while females that hear song during development are choosier than females lacking acoustic experience (Bailey and Zuk 2008).

I tested the role of acoustic experience in modulating two parameters: perceived sperm competition risk and aggressive behavior (*sensu* DiRienzo et al 2012). As noted above, sperm competition risk refers to the likelihood that one males' ejaculate will compete with that of another male to fertilize ova (Parker 1970), and theory predicts that males should invest more in the ejaculate as sperm competition risk increases (Parker et al 1997, Simmons 2001). Males may assess risk via direct indicators, such as physical interaction with potential rivals (Bretman et al 2010, Barbosa 2012, reviewed in Wedell et al 2002), as well as indirect indicators, such as visual or chemical cues from conspecifics (Bretman et al 2011a). However, relatively little is known about the role that acoustic cues may play in modulating perceived sperm competition risk (though see Bretman et al 2011b). I sought to partially address this gap in the literature by determining whether males could use acoustic cues to assess the risk of sperm competition and adjust ejaculate expenditure accordingly.

As noted above, acoustic experience can modulate perceived population density. Male crickets in particular are sensitive to changes in density, responding with varying levels of aggression. In *G. bimaculatus*, males reared at low density are more aggressive than males reared at higher densities (Iba et al 1995, Nagamoto et al 2005). Further, male *G.*

integer reared in song-dense environments, like one that would be experienced in a high-density population, were less aggressive than those reared in silent environments (DiRienzo et al 2012). Because the flatwing mutation alters the acoustic environment, I investigated whether males that were reared in song-dense environments differed in levels of aggression from males reared in silent environments.

Situation 2) Multimodal signal compensation

Communication in crickets is multimodal, with recent evidence indicating that courtship song and cuticular hydrocarbons (CHCs), long-chain waxy substances found on the exoskeleton and thought to be initially evolved in insects to resist desiccation (Blomquist et al 1998), function to send multiple messages about different aspects of mate quality (Simmons et al, in press). In some other species utilizing multimodal communication, signals can serve as backups to one another when one modality is temporarily blocked or unavailable (Candolin 2003, Hebets and Papaj 2005). For example, wolf spiders, which use visual and vibratory signals, switch modality depending on the environment, using more visual signals when on substrates that preclude transmission of vibrations (Gordon and Uetz 2011). However, little is known about potential signal compensation if a signal modality is permanently as opposed to temporarily lost.

Because the study species uses multimodal communication, there is a potential avenue for signal compensation to operate. Crickets utilize at least two major communication modalities: acoustic signals and chemical cues. Acoustic signals are found in the form of multiple song types (Alexander 1962) - long-range calling song to attract mates (Zuk et al 1993, 2008), short-range courtship song that provides potential mates with information about caller quality (Rebar et al 2009), and a song used in male-male aggression (Alexander 1961, Logue et al 2010). Chemical cues come in the form of CHCs, which in this species, provide conspecifics with information about the sex and mating status of other individuals (Thomas and Simmons 2008, 2010). CHCs are limited to contact chemoreception, but can be deposited on substrates and detected by other individuals (Bailey 2011). Given that flatwings lack the ability to produce song, I sought to investigate whether they compensate by increasing the use of chemical cues to secure mating opportunities.

Situation 3) The maintenance of multiple male morphologies

The existence of multiple male morphologies presents an interesting situation, for if one male morph is better at securing mates and reproducing, it should be favored in the population, and cause the disappearance of the other morphologies (Darwin 1874). One explanation for the maintenance of multiple male morphologies is negative frequency-dependent selection, in which rare males experience a fitness advantage (Sinervo and Lively 1996). This is the case with populations of the side-blotched lizard *Uta stansburiana*, in which three male morphs are maintained via a natural 'rock-paper-scissors' situation (Sinervo and Lively 1996). In contrast, Darwin postulated that multiple male morphologies could only be maintained if they experienced equal lifetime fitness (Darwin 1874, Schuster and Wade 2003). On the island of Kauai, flatwings represent ~90% of the male population (Zuk et al 2006), and this proportion has remained at this level for several years (M. Zuk, unpublished data)

Flatwing males are unable to attract mates via the production of calling song (Zuk et al 2006). If these males cannot secure matings as easily, how are they maintained in the population? One answer may be a tradeoff between daily reproductive rate and overall lifespan. For example, in *Mnais* damselflies orange-winged territorial males have high daily mating rates, but do not live as long as clear-winged males (Tsubaki et al 1997). These clear-winged males compensate for a lower daily reproductive rate by living longer, and so the two morphs experience equal lifetime reproductive success (Tsubaki et al 1997).

There is good evidence that calling males suffer increased mortality and a concomitant reduction in mean lifespan as a result of parasitization from *O. ochracea*. In a comparative study of three species of crickets, Murray and Cade (1995) found that in species not subject to parasitism, males either do not differ in age from or are older than females. However, in a species subject to parasitism from *O. ochracea*, males are younger than females (Murray and Cade 1995). In *T. oceanicus*, males from two populations without *O. ochracea* are older than females, but in a third population subject to parasitism, males did not differ in age from females (Simmons and Zuk 1994). Importantly, females from all three populations did not differ in age, indicating that male age in parasitized populations was reduced relative to male age in unparasitized locales (Simmons and Zuk 1994).

Because flatwing males are unable to be located by *O. ochracea*, they should be have a mean age greater than that of normal-winged males. This would give flatwings a longer 'operational reproductive lifespan' (Tsubaki et al 1997) with which to secure additional matings and increase reproductive success. I tested this by surveying the ages of field-caught individuals using a standardized technique of counting daily growth layers found in the cuticle of crickets (Neville 1963, Zuk 1987, Simmons and Zuk 1994).

Summary

In short, evolutionary events do not occur in a vacuum. Rather, they can generate a cascade of effects that influence a number of parameters, from ecology (Bassar et al 2010) to physiology and behavior (Bailey et al 2010, Kasumovic and Brooks 2011). In the following chapters, I take advantage of the evolutionary loss of a sexual signal that provides important social information to conspecifics in order to elucidate some of the potential consequences of evolutionary events.

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Chapter 1

Acoustic cues alter perceived sperm competition risk in the field cricket *Teleogryllus oceanicus*

Brian Gray[a,] and Leigh W. Simmons[b]

a. Department of Biology, University of California, Riverside, Riverside, CA 92521, USA

b. Centre for Evolutionary Biology, School of Animal Biology (M092), The University of Western Australia, Crawley 6009, Australia

ABSTRACT

Sperm competition game theory predicts that males should respond to increasing sperm competition risk by increasing ejaculate expenditure. There is considerable support for this prediction from a diverse range of taxa. However, the cues males use to assess risk and the fitness returns for strategic ejaculation are less well understood. We explored the role of acoustic cues in the assessment of sperm competition risk by manipulating male experience of acoustically signaling conspecifics in the field cricket *Teleogryllus oceanicus*. Compared with males reared in acoustic isolation, males reared in song-dense environments mimicking a high sperm competition risk produced ejaculates with a greater percentage of viable sperm. However, acoustic experience had only a weak and nonsignificant effect on competitive fertilization success. We argue that female influences on paternity are likely to have a strong moderating effect on male fitness returns from prudent allocation and call for more studies that address the con- sequences of strategic ejaculation for male fitness.

INTRODUCTION

Sperm competition game theory predicts that males should allocate their ejaculates prudently, with male expenditure increasing with the risk of sperm competition (Wedell et al. 2002; Parker and Pizzari 2010). That is, because the production of ejaculates can be costly (Dewsbury 1982; Simmons 2001), males experiencing increased sperm competition risk should produce more competitive ejaculates, whereas those experiencing little or no risk should reduce their expenditure. Empirical data generally support this theoretical expectation. Recent meta-analyses of the literature found moderate and significant general effects of sperm competition risk on ejaculate expenditure, and these effects were prevalent across multiple taxa (delBarco-Trillo 2011; Kelly and Jennions 2011). Less is known about the cues that males use to assess sperm competition risk or, more importantly, how adjustments in male expenditure translate into fertilization success.

Males may use the physical presence of rival conspecifics as an indicator of risk. For example, researchers often alter either the number of rivals to which a focal male is exposed immediately before or during mating (Ramm and Stockley 2007; Bretman et al. 2010; Barbosa 2012; reviewed in Wedell et al. 2002) or alter the operational sex ratio (Allen et al. 2011; reviewed in Simmons 2001). However, in a number of organisms, such as solitary species, males neither mate in the presence of competitors nor regularly interact with one another; as such, the males of these species must estimate sperm competition risk using sociosexual cues other than the physical presence of rivals. A growing body of literature suggests that social cues experienced during development may have long-term effects on growth and behavior (reviewed in Kasumovic and Brooks 2011). Recent evidence indicates that males may utilize visual, odor, or acoustic cues to assess sperm competition risk (reviewed in Bretman, Gage, et al. 2011), and empirical support for prudent ejaculate allocation in the presence of such cues exists for a number of taxa. Male meadow voles ejaculate more sperm when mating in the presence of conspecific male odors (delBarco-Trillo and Ferkin 2004, 2006; though see Ramm and Stockley 2009), and in the butterfly, *Pieris napi*, males produce larger ejaculates when mating in the presence of the male sex pheromone citral (Larsdotter Mellström and Wiklund 2009). In humans, men viewing sexually explicit images involving other men produced ejaculates with a greater percentage of motile sperm than men viewing images of women alone (Kilgallon and Simmons 2005). Finally, in *Drosophila melanogaster*, males require at least 2 of acoustic, visual, tactile, and olfactory cues to respond to the presence of rival sperm competitors (Bretman, Westmancoat, et al. 2011). With the exception of Bretman, Westmancoat, et al. (2011), these studies have focused on the use of olfactory or visual cues used by males in assessing risk. To date, the role of acoustic cues in modulating perceived risk remains largely unstudied. Acoustic cues are likely to be important, given that they represent a major sensory modality in a number of species, including insects, anurans, birds, and mammals.

In crickets, acoustic cues can provide information to juveniles about local competition and lead to changes in growth and adult behavior (reviewed in Kasumovic and Brooks 2011). In *Gryllus integer*, males reared in silence are more aggressive and more likely to become dominant than congeners reared in song-dense environments (DiRienzo et al. 2012). In the field cricket *Teleogryllus commodus*, the presence and quality of song affects a number of developmental traits. Males exposed to acoustic environments indicating low levels of competition matured faster but attained a smaller body size, while investing more in calling effort (Kasumovic et al. 2011). Further, males reared in low density environments lived longer than males experiencing higher densities (Kasumovic et al. 2012). Similarly, in our study species, Teleogryllus oceanicus, changes in the acoustic social environment affect a suite of morphological and behavioral traits in both males and females (Bailey and Zuk 2008; Bailey et al. 2008, 2011). Specifically, the presence or absence of song affects male growth and behavioral decisions. Males reared in song-dense environments grow larger, invest more in reproductive tissues, and are less likely to act as satellites than males reared in song-free environments (Bailey et al. 2010).

Given the role of acoustic cues in altering developmental trajectories and behaviors, we investigated whether acoustic cues can be utilized to gauge sperm competition risk using the Australian field cricket, T. oceanicus. This species has been the subject of a number of studies on ejaculate quality and sperm competition risk. Sperm viability (the percentage of live sperm within an ejaculate), rather than sperm number, is a significant predictor of paternity share when females mate multiply (Simmons et al. 2003; García-González and Simmons 2005a), and males of this species modify their expenditure on sperm viability with changing sperm competition risk and intensity (Simmons et al. 2007). Simmons et al. (2007) manipulated risk by allowing males to make physical contact with conspecific rivals. Males of this species appear to respond to contact chemoreceptive cues, reducing ejaculate expenditure in response to male-derived cuticular hydrocarbons (CHCs) left on the female's cuticle after copulation (Thomas and Simmons 2007). However, the primary signaling modality in this species is acoustic, and it is not presently known whether males can utilize acoustic cues alone to assess sperm competition risk. We predicted that males exposed to acoustic cues will perceive a greater risk of sperm competition than males that do not experience acoustic cues and respond to acoustic cues by producing ejaculates with greater sperm viability.

As noted above, few studies have examined how strategic adjustments in ejaculate expenditure translate into male reproductive fitness (Bretman, Fricke, et al. 2009; Barbosa 2012). In many species, including *T. oceanicus* (Simmons and Beveridge 2010), females can exert considerable control over which sperm they utilize when fertilizing their ova (Eberhard 1996), so that male tactics in sperm competition may not always result in increased male fitness. We, therefore, also estimated the effect of strategic ejaculation on male fitness by determining the paternity of offspring produced by females mating with males that had experienced different acoustic environments prior to mating.

METHODS

General rearing

Crickets used in all trials were taken from a stock population derived from a banana plantation in Carnarvon, Western Australia.The population consists of several thousand outbred individuals and is supplemented annually with new individuals collected from the same plantation. Males were selected as late-instar juveniles and reared in constant-temperature growth chambers on a 12:12h light:dark cycle at 28°C.

Males were placed individually into small plastic containers (7×7 × 5cm), given cat food and water *ad libitum*, and isolated visually from one another by covering the outer walls of each container with opaque paper. Individual containers were cleaned weekly, and males were checked at least 5 times per week for adult eclosion. On eclosion, males were silenced by removal of the plectrum with small scissors, a procedure that does not affect male behavior (Bailey et al. 2008).

Individual males were randomly assigned to 1 of 2 acoustic treatments. In the “no-song” treatment, males were housed in their individual containers within 1 of 2 growth chambers without any singing individuals. In the “song” treatment, males were housed in their individual containers within 1 of 2 growth chambers that also contained approximately 100 singing individuals. Thus, 2 replicate growth chambers were used within each treatment. This method has been used previously to study the effect of acoustic experience on behavioral, morphological, and physiological traits in this species (Bailey and Zuk 2008; Bailey et al. 2010, 2011; Thomas et al. 2011).

Sperm viability assays

Sperm viability assays were performed using published methods 14–16 days post-adult eclosion (García-González and Simmons 2005a; Simmons 2012). Fresh spermatophores (within 2h of production) were ruptured in 20 µL of Beadle saline (128.3mM NaCl, 4.7 mM KCl, and 23 mM CaCl2) by severing the evacuation tube of the spermatophore with fine scissors. Five microliters of the sperm–saline solution were mixed with 5 µL of 1:50 diluted 1mM SYBR-14 (Molecular Probes) and incubated in the dark for 10min. Two microliters of propidium iodide were then added and the sample incubated in the dark for another 10min. SYBR-14 stains live sperm green, whereas propidium iodide stains dead sperm red. Sperm were observed at ×20 magnification using fluorescence microscopy. Five hundred sperm were scored in each sample, and all assays were performed blind to experimental treatment.

Paternity assay

We took advantage of a morphological marker (white eyes) to determine paternity (Simmons et al. 2003). White-eyed males and females were isolated from laboratory stocks as late-instar juveniles. These were then housed in single-sex containers with 6–10 other individuals. Containers were checked at least 5 times weekly and newly emerged adults removed to single-sex containers with other similarly aged adults. Containers were housed in the same constant-temperature room as the general stock population, so that all white- eyed (*we*) crickets were exposed to singing males.

We utilized a protocol adapted from García-González and Simmons (2005a) to assign paternity. Old spermatophores were removed from *we* males before exposing them to females, thus ensuring the production of a fresh spermatophore during mating. A virgin *we* female was mated first to a *we* male. Matings were observed to ensure successful spermatophore transfer and retention by the female. Males were allowed to mate guard for 45min, preventing female removal of the spermatophore. The female was then mated with a wild-type black-eyed male from 1 of the 2 acoustic experience treatments. Again, males were left to guard females and prevent spermatophore removal until 45 min had elapsed. Each cricket was used once only.

After completing both matings, females were placed individually into a container with food and water *ad libitum*, as well as damp cotton wool for oviposition. Females were allowed to oviposit for 21 days. All hatchlings from each female were scored for eye color. Scoring of hatchlings was performed blind to the experimental treatment. We scored eye color for a total of 34 493 offspring, distributed across 62 double-mated females (mean offspring per female = 572, range 14–1303).

Experimental males were weighed to the nearest 0.1mg. Pronotum width was measured using digital calipers to the nearest 0.01 mm.

Statistical analyses

Sperm viability was analyzed using a generalized linear model (GLM) with a binomial error and logit-link function with treatment as the main effect, the number of live sperm contained in the sample as the dependent variable, and the total sperm counted as the binomial denominator. Biologically relevant measures (total body mass, pronotum width, soma mass, testes mass, and accessory gland mass) were entered as additional factors. Because of overdispersion, F-tests were used rather than chi-square tests (Crawley 1993). Nonsignificant terms were removed from the final model. There was no effect of the different replicate rooms used within each treatment on any dependent variables (see Results), so data were pooled across rooms for analyses.

Reproductive success was analyzed in the same manner as sperm viability, with treatment as the main effect, the number of wild type offspring as the dependent variable, the total number of offspring counted as the binomial denominator, and biologically relevant measures, with the addition of mating interval, included as additional factors. Again, nonsignificant terms were removed from the final model.

GLMs for sperm viability and reproductive success were per- formed in GLMStatX, and all other analyses performed in JMP 9.0. Sperm viability and paternity data are presented as mean percentages ± 1 standard error of live sperm and offspring sired, respectively.

Cohen’s *d* and associated 95% confidence intervals were calculated following Nakagawa and Cuthill (2007).

RESULTS

Sperm viability

We analyzed the song and no-song treatments separately to examine whether there was an effect of replicate rooms on sperm viability. There were no differences between replicate rooms within the song ($F_{1,45} = 6.32 \times 10^{-2}$, $P = 0.803$) or no-song treatments ($F_{1,48} = 0.105$, $P = 0.747$), so we pooled the data across replicate rooms within each acoustic treatment. Males reared in the song environments produced ejaculates with a greater proportion of viable sperm than males reared in song-free environments ($F_{1,95} = 4.397$, $P = 0.039$; effect size, Cohen’s $d = 0.425$, 95% CI = 0.181–0.832; Figure 1).

Paternity

As with sperm viability, we assessed whether there was an effect of replicate rooms within treatments on paternity. We did not find an effect of replicate rooms on paternity within the song ($F_{1,27} = 0.189$, $P = 0.667$) or no-song treatments ($F_{1,31} = 5.743 \times 10-2$, $P = 0.812$), so we pooled the data across replicate rooms within each treatment. Males reared in song environments did not sire more offspring than males reared in song-free environments ($F_{1,60} = 4.189 \times 10-2$, $P = 0.839$; effect size, Cohen’s $d = 0.187$, 95% CI = −0.320–0.694; Figure 1).

DISCUSSION

It is now widely recognized that males may allocate their ejaculates prudently, depending on their perceptions of sperm competition risk (delBarco-Trillo 2011; Kelly and Jennions 2011). Although males are known to respond to changes in the operational sex ratio and the physical presence of rivals during mating, little is known about the specific sociosexual cues that males use to detect the presence of rivals (Bretman, Gage, et al. 2011; Bretman, Westmancoat, et al. 2011). In our study, we found that males reared in song-dense environments produced ejaculates with greater sperm viability, indicating that males use acoustic cues to detect the risk of sperm competition and increase their expenditure on the ejaculate as predicted by sperm competition game theory (Parker and Pizzari 2010).

Studies of strategic ejaculation by males assume that male responses to sperm competition risk serve to increase male fitness in the face of competition. Few studies have actually tested this assumption, though it is supported in the fruit fly *D. melanogaster* (Bretman et al. 2010; Bretman, Westmancoat, et al. 2011) and the soldier fly *Merosargus cingulatus* (Barbosa 2012). It is notable, therefore, that the effect of acoustic cues on a male's competitive fertilization success in our study was not statistically significant. Males exposed to calling conspecifics did achieve a greater average paternity share than those reared in acoustic isolation, but the effect size was 44% smaller than that for sperm viability. The effect sizes showed considerable overlap, so we are unable to conclude that males do not increase their fitness via increased expenditure on the ejaculate. We note that the variance in paternity within treatment groups was much greater than the variance in sperm viability.

The greater variance in paternity share has both statistical and biological implications for interpreting the results obtained. Statistically, the greater variance, coupled with lower effect size, reduces our statistical power to detect a statistically significant effect of acoustic environment on paternity. Biologically, we expect lower effect sizes for 2 reasons. First, unlike sperm viability, measures of paternity are made relative to a male competitor. Because male crickets show innate, genetically based variance in sperm viability (Simmons and Roberts 2005), random variation in the different we competitors assigned to each treatment male will have introduced variance into our estimates of individual sperm competitive success; some males will have competed against a poor competitor and obtained high paternity scores, whereas others will have competed against a good competitor and obtained low paternity scores, despite their absolute investments in sperm viability (García-González 2008). Second, as Parker (1970) noted, we cannot expect females to be an inert environment within which males play out their battles. Female *T. oceanicus* appear to have considerable control over whether they store and utilize a particular male's sperm. Females collected from natural populations store sperm from many more males than they actually utilize during fertilization (Simmons and Beveridge 2010). Moreover, females of this and other cricket species have been shown to bias paternity toward unrelated males and do so by preferentially storing sperm from unrelated males (Simmons et al. 2006; Tuni et al. 2013; see also Bretman, Newcombe, et al. 2009). In the closely related species *T. commodus*, females similarly bias paternity toward more attractive males (Hall et al. 2010). Thus, despite strategic adjustments in ejaculate expenditure by males, females ultimately determine a male's fertilization success. Male-by-male and male-by-female interactions are likely to be important in natural populations, so that male fitness returns from prudent allocation may generally be smaller than our observations of adjustments in sperm numbers and/or ejaculate quality might suggest. More studies are required that actually assess the fitness returns associated with strategic ejaculate allocation by males, because it is only the magnitude of fitness benefits that will inform us of the strength of selection favoring this particular form of phenotypic plasticity.

How males modulate sperm viability is currently unknown, but evidence indicates that seminal fluid adjustment is the most likely mechanism. The viability of sperm from a given male of this species can be increased by mixing its sperm with the seminal fluid from another male with higher sperm viability (Simmons and Beveridge 2011), suggesting that proteins within the seminal fluid serve to nourish, protect, and/or activate sperm. Recently, Simmons et al. (2013) identified 21 novel seminal fluid proteins, many of which are implicated in affecting sperm function. Research on *D. melanogaster* has shown that males are constrained in their ability to manufacture and transfer seminal fluid proteins (Sirot et al. 2009), and males will adjust the amounts of seminal fluid proteins contained in their ejaculate in response to sperm competition risk (Sirot et al. 2011). Sperm viability only affects the fertilization success of male *T. oceanicus* when in competition with other males (García-González and Simmons 2005a, 2005b), so that males may conserve costly seminal fluids for use when the risk of sperm competition is high.

Sperm viability appears to be remarkably sensitive to social experience in *T. oceanicus*. Thomas and Simmons (2009a) examined the relationship between male dominance and ejaculate quality, find- ing that subordinate males produce ejaculates with lower sperm viability and lower competitive fertilization success. Intriguingly, subordinate males also appear to upregulate their production of CHCs (Thomas and Simmons 2009a, 2011). CHCs serve as contact pheromones that females find attractive (Thomas and Simmons 2009b), and their expression is negatively associated with sperm viability, suggesting that these 2 traits might be subject to an allocation trade-off. Subordinate males also adopt a silent satellite strategy when searching for mates in order to avoid detection by dominant males (Burk 1983) and may utilize CHCs as an alternative, albeit costly, means of securing matings. In their study, Bailey et al. (2010) found that male T. oceanicus reared in silent environments were more likely to adopt satellite behavior, and Thomas et al. (2011) found that the males reared in acoustic isolation in this study invested more heavily in CHC signaling than those reared in acoustically dense environments.

Collectively, these findings suggest that strategic increases (decreases) in ejaculate quality when faced with varying sperm competition risk may be associated with reductions (increases) in male expenditure on precopulatory odor signals that promote mating success. Negative associations between male investment in pre- and postcopulatory reproductive expenditure are central to sperm competition game theory (Parker et al. 2013).

In conclusion, we show that male crickets use acoustic cues to assess sperm competition risk and increase ejaculate expenditure when they perceive calling rivals in their environment. Although the effects of acoustic cues on male ejaculate expenditure were strong, their consequences for competitive fertilization success were considerably weaker and nonsignificant. We suggest that the considerable female effects on paternity in this species limit a male's ability to monopolize paternity with polyandrous females. The evolutionary loss of acoustic signaling in some populations of T. oceanicus in the Hawaiian archipelago (Zuk et al. 2006) has resulted in a change in the acoustic environment of this species, such that acoustic signals, or the lack thereof, no longer provide males with a reliable indicator of sperm competition risk. As such, we predict that in these populations, we should see an evolutionary loss of ejaculate plasticity in response to acoustic cues and perhaps an increased reliance on alternative cues, such as visual, tactile, and/or olfactory cues.

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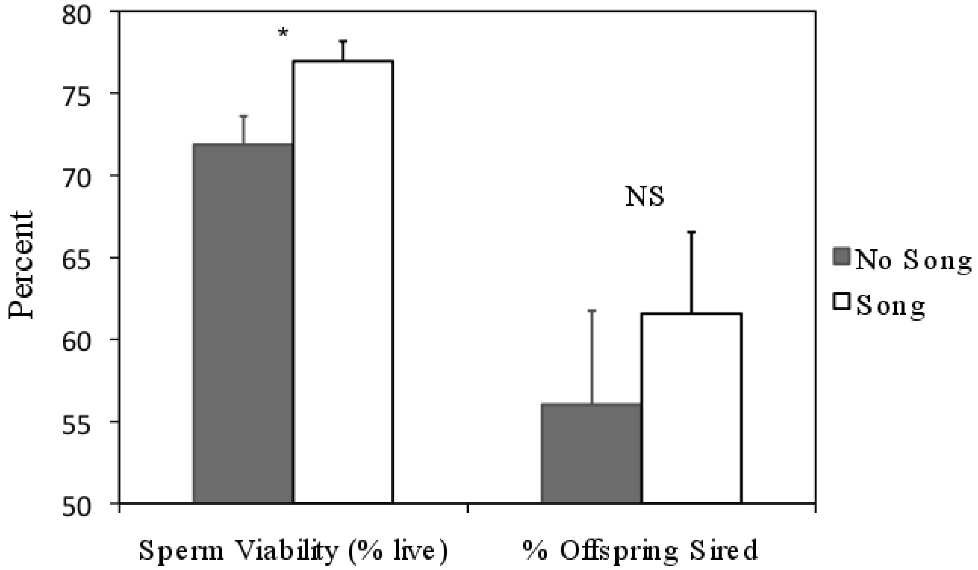

Figure 1.1 Sperm viability and reproductive success (percentage of offspring sired) with respect to rearing environment. The “song” environment represents a greater perceived risk of sperm competition, whereas the “no-song” environment represents a low perceived risk of sperm competition. Bars represent standard errors.

Chapter 2

Acoustic experience influences aggression in the field cricket, *Teleogryllus oceanicus*

Brian Gray[1], Deborah De La Riva[2], and Marlene Zuk[1,3]

1. Department of Biology, University of California, Riverside

2. Department of Entomology, University of California, Riverside

3. Department of Ecology, Evolution, and Behavior, University of Minnesota

ABSTRACT

Social experience may have a number of behavioral and physiological consequences. Social experience may derive from direct encounters with other individuals, or may be gleaned from cues in the environment that provide information about local population density and intraspecific competition. In the field cricket *Teleogryllus oceanicus*, the acoustic cues used to provide social information have been lost in some Hawaiian populations, thereby changing the social environment experienced by all individuals within these populations. Male crickets reared in song-dense environments, such as those that would typically be encountered in high-density populations, were less aggressive than males reared in silent environments, supporting previous work in other species of crickets that indicates the social environment experienced by individuals can alter behavioral trajectories. The propensity to move first was associated with an increased likelihood to initiate agonistic encounters, suggesting a potential behavioral syndrome in male *T. oceanicus*. The acoustic environment did not affect this association, and males from song-dense environments were as likely to win agonistic encounters as males from silent environments.

INTRODUCTION

Social cues experienced by juveniles and adults can influence investment in a number of physiological and behavioral traits (Kasumovic and Brooks 2011). For example, population density affects the level of aggression displayed by male crickets *Gryllus bimaculatus*, with males from high-density populations less aggressive than those from low-density populations (Iba et al 1995, Nagamoto et al 2005). For species that do not live in dense aggregations, other social cues may be used to estimate local population density, such as sexual signals. Sexual signals are used by a wide range of taxa (Andersson 1994), and given that many sexual signals (e.g. mate advertisement calls) have evolved to attract the attention of prospective mates, these signals may also provide conspecifics with information about local population parameters.

In crickets, males produce sexual signals in the form of calling and courtship songs (Alexander 1962). More generally, acoustic cues seem to be particularly important conveyors of social information. Indeed, a number of studies have documented changes in physiology and behavior in both male and female crickets as a result of acoustic experience (*T. oceanicus*: Bailey and Zuk 2009, Bailey et al 2010, Bailey et al 2011, Thomas et al 2011, Gray and Simmons 2013; *T. commodus*: Kasumovic et al 2011, Kasumovic et al 2012). The recent loss of a sexual signal in Hawaiian populations of the field cricket *T. oceanicus* (Zuk et al 2006) generates a novel acoustic environment for both juvenile and adult crickets. Hawaiian *T. oceanicus* are subject to an acoustically-orienting parasitoid fly (Zuk et al 1993), and parasitized males typically die 7-10 days post-infestation (Adamo et al 1995). On the islands of Kauai and Oahu, males have evolutionarily lost the wing structures necessary to produce sound (Zuk et al 2006). These males, called *flatwings*, represent more than 90% of the male population of Kauai and approximately 50% of the males on Oahu (M. Zuk, unpublished data). Populations with large proportions of flatwings are relatively silent, so juvenile crickets from these populations may experience an acoustic environment very different from that experienced by conspecifics in other populations (Bailey et al 2010).

Acoustic experience may provide conspecifics with information about population density, with acoustically-dense environments signalling a population with increased male-male competition. Studies on the cricket *G. bimaculatus* have found that increased density leads to a reduction in aggressive behavior (Iba et al 1995, Nagamoto 2005) and that tactile cues can influence aggression (Adamo and Hoy 1995). Further, in the cricket *Gryllus integer*, acoustic experience alone alters levels of aggression. Males from silent environments, presumably reflecting a lower population density, display more aggressive behavior than males from song-dense environments (DiRienzo et al 2012). In contrast to *T. oceanicus,* in *G. integer* the acoustic environment is seasonally variable. *G. integer* produces one brood annually, so the first crickets to hatch experience a silent acoustic environment. Crickets hatching later in the season hear the calling songs of the early-developing crickets. DiRienzo et al (2012) suggest that reduced aggression in song-dense environments may be a way for crickets to reduce the costs of male-male competition, which may be greater later in the season when more males are sexually mature. Given the loss of a sexual signal and the concomitant change in acoustic environment associated with the flatwing mutation, we sought to investigate the role of acoustic experience in modulating levels of aggression in *T. oceanicus.* Despite the differing causes for modified acoustic environments in *T. oceanicus* and *G. integer*, the acoustic outcomes are functionally equivalent. Accordingly, we predicted that crickets reared in silent environments, mimicking a low perceived population density, would be more aggressive than those reared in song-dense environments. Because *T. oceanicus* breeds continuously (Tinghitella and Zuk 2009), there are several overlapping generations in the field at any given time. While this is unlikely to matter on Kauai and the Big Island, which are both dominated by a single male morph, on Oahu, flatwings and normal-winged males exist in roughly equal numbers (M. Zuk, unpublished data). This may lead to variation in male acoustic experience, with some hearing several calling males and others experiencing near-silence during development. To determine whether acoustic experience affected the probability of winning an aggressive interaction, we allowed one male from each acoustic environment ("across-environment interactions") to interact in pairwise encounters. Because we predicted that crickets from silent environments would be exhibit more aggression than individuals from song-dense environments, we also predicted that males reared in silent environments would be more likely to win encounters against crickets from song-dense environments .

In many species of cricket, aggression is linked to reproductive success, with males successful in aggressive encounters more likely to successfully obtain mates (Cade 1979, Kortet and Hedrick 2005), and females preferring the chemical cues of dominant males (Kortet and Hedrick 2005; though see Thomas and Simmons 2009). Further, aggression may correlated with other behaviors, forming a behavioral syndrome (Sih et al 2004). Aggression is a costly endeavor that involves the risk of serious injury or death (Jaeger 1981, Marler and Moore 1988), so individuals willing to exhibit aggression may be more 'bold' and more likely to engage in other risky behaviors (Kortet and Hedrick 2007). Only recently have studies started to address how social experience might affect observed correlations between behaviors (Lihoreau et al 2009, Aragón 2011, Sih 2011). While correlations between behaviors have been observed in other crickets (Hedrick 2000, Kortet and Hedrick 2007), it is unclear whether any behavioral syndromes exist in our study species. As such, we sought to examine whether such behavioral consistencies exist in our study species, and how these were affected by acoustic experience. We tested male crickets for an 'activity/aggressiveness syndrome' (Kortet and Hedrick 2007), which here describes a relationship between two risky endeavors: latency to move after a brief disturbance (removal of a divider; see methods) and the initiation of agonistic interactions. In *G. integer*, males that began moving first when placed in a novel environment were more aggressive (Kortet and Hedrick 2007). We also investigated whether acoustic experience affected this relationship. Because males experiencing song-dense environments may be less aggressive than those reared in silent environments, we predicted that there would be a stronger relationship in crickets from no-song environments between a willingness to initiate aggressive behaviors and a propensity to be the first to begin moving following a disturbance.

METHODS

General Rearing

Crickets were taken from a large, outbred laboratory stock originating from Oahu and supplemented annually. Crickets were isolated as late-instar juveniles as soon as sex differences were apparent and reared individually in 118ml containers at 25°C and on a 12:12 light:dark cycle. Crickets were given a piece of cardboard egg crate for shelter, as well as *ad libitum* Purina rabbit chow and a cotton-plugged vial of water. Food and water were replaced twice weekly, and crickets checked for adult eclosion daily.

Crickets were randomly assigned to one of two acoustic treatments: song or no song. Complete methodological details regarding the establishment of the acoustic environments can be found in Bailey and Zuk (2008). Briefly, crickets in the song environment were reared in a Precision 818 incubator outfitted with speakers playing multiple Oahu calling song models throughout the dark phase of the photoperiod. Sound pressure levels were ~70dB at the position of the crickets. Crickets in no song environments were reared in identical incubators, but without calling song playback. We rotated the environments among incubators to prevent incubator effects.

To maintain a silent environment, a small wing structure was removed with dissecting scissors from the forewings of each male cricket. Crickets in the song environment were treated identically to control for any possible effects of handling, though this procedure does not appear to affect male behavior (Bailey et al 2008). All crickets were used only once.

Experiment 1: Within-Environment Interactions

Body mass may affect aggressive behavior in crickets (Hofmann and Schildberger 2001, Savage et al 2005). To mitigate this in our experiment, males were matched by body size by ensuring that pairs did not differ in mass by more than ten percent. Males were also matched by age, such that all crickets were 24-26 days post-eclosion during aggression trials. Pairs were taken from the same acoustic environment, so that both males in a trial had the same acoustic experience.

Two days prior to testing, male crickets were placed in a 30x20x20cm (L x W x H) plastic testing arena with a removable opaque divider along the short axis and a sand substrate. One male was housed on each side of the container, and each side had a dish of Purina rabbit chow, a piece of cardboard egg crate, and a cotton-plugged vial of water. Crickets were marked on the pronotum with liquid paper for identification purposes.

On the day of the trial, the food, water, and shelter were removed prior to testing. Testing took place in a darkened room (temp = 25±1.5°C) outfitted with red light for observations. Trials were recorded using a tripod-mounted Sony DCR-200 digital video recorder set to the 'night vision' mode. The divider was removed and timing began. We recorded trials for 20 minutes after the two crickets came into physical contact with one another. Between trials, arenas were cleaned with 100% ethanol and the sand substrate replaced to minimize the presence of any residual pheromones. Upon completion of the trials, crickets were placed back into the general population.

Videos were scored following a protocol adapted from Logue et al (2010). Aggressive behaviors in crickets are highly distinctive and easily recognized (Alexander 1961). Following protocols adapted from Logue et al (2010) and DiRienzo et al (2012), we recorded the number of stridulations, aggressive acts, initiations, and retreats performed by each individual (see Table 1 for definitions). We also recorded who won each encounter. Winners often chase their opponent after an encounter and stridulate aggressively, while losers typically avoid winners for the remainder of the trial. All trials were scored blind to the acoustic treatment.

Experiment 2: Across-environment interactions

To determine if acoustic experience affected the likelihood of winning an encounter, we generated pairs of age- and size-matched males using the criteria above, though males in this experiment were 8-10 days post-adult eclosion during trials. In this experiment, one male in each pair came from the song environment, while the other came from the no-song environment.

On the day of each trial, crickets were removed from their 118ml containers, marked on the pronotum with liquid paper for identification, and placed into a testing arena measuring 13x13x18cm with an opaque divider along the diagonal (Judge and Ting et al 2010). One male was placed on each side of the divider and left to acclimate for ten minutes. The divider was then removed and the males allowed to interact for ten minutes after making first contact. Trials were again performed in a darkened room at 25±1.5°C equipped with red light for observation. All trials were video-recorded and arenas cleaned between trials as noted above.

Videos of these trials were scored for the identity of the cricket that moved first following the removal of the divider, that initiated each encounter, and that won encounters. Videos were scored without knowledge of the acoustic experience of each crickets.

Data analysis

Data were transformed when appropriate. Data were analyzed in JMP 10.0.0 (SAS Institute, Cary, NC). All data are presented as means ± se. Binomial tests reported below are one-tailed.

RESULTS

Experiment 1

Males that moved first were more likely to initiate the first aggressive encounter (binomial test; N = 43, p = 0.033), but not more likely to win the encounter (binomial test; N = 43, p = 0.111). Males that initiated encounters, regardless of whether or not they were the first to move, showed a tendency to win encounters, but this difference was not statistically significant (binomial test; N = 43 p = 0.063). Aggression was not influenced by body mass. Winners did not differ in body mass from losers (Figure 1; mean winner mass = 0.518 ± .008g, mean loser mass = 0.510 ± .009 g, t = 0.508, df = 41, p = 0.614), and mass was not associated with the total number of aggressive acts performed by winners (R^2 = 0.041, df = 43, p = 0.195) or losers (R^2 = 0.001, df = 43, p = 0.815).

Pairs reared in no song environments displayed greater summed aggression than pairs from song environments (Figure 2; mean number of acts, no song environment = 52.8 ± 5.2 acts, song environment = 39.5 ± 5.8 acts, t = -1.837, df = 41, p = 0.037). However, acoustic environment did not affect the number of summed stridulatory bouts performed by pairs (t = -0.655, df = 41, p = 0.258).

Experiment 2

Males that moved first were more likely to initiate (binomial test; N = 44, p < 0.001), and win aggressive encounters (binomial test; N = 44, p = 0.002). Males that initiated encounters, regardless of whether or not they were the first to move, were more likely to win the encounters (binomial test; N = 44, p < 0.001).

Winners did not differ from losers in body mass (mean winner mass = 0.590 ± .085g; mean loser mass = 0.591 ± .086g; t = 0.268, df = 43, p = .790). Males from silent environments were no more likely than crickets from song environments to move first (binomial test; N = 44, p = 0.146), initiate a battle (binomial test; N = 44, p = 0.087) or win battles (binomial test; N = 44, p = 0.559).

DISCUSSION

A growing body of literature highlights the importance of the social environment as a modulator of phenotypic traits. Here, we demonstrate that one component of the social environment - acoustic cues - can influence the expression of aggressive behavior in *T. oceanicus*, likely by changing perceptions of local population density. Consistent with our predictions and with the results of DiRienzo et al (2012) on *G. integer*, males reared in silent environments were more aggressive than males from song-dense environments. Acoustic cues in *T. oceanicus* appear to convey information about population density (Bailey et al 2010, Bailey et al 2011, Gray and Simmons 2013), making our findings consistent with what is known about the role of population density in modulating aggressive behavior in the closely related *G. bimaculatus* (Iba et al 1995, Nagamoto et al 2005).

However, in contrast to DiRienzo et al (2012), we did not find an effect of acoustic experience on aggressive stridulation. While male *G. integer* exposed to song aggressively stridulate less than males not exposed to song, male *T. oceanicus* from song and no-song environments did not differ in the number of bouts of aggressive stridulation performed during trials. This may be due to a difference in methodology; DiRienzo et al (2012) utilized males that were able to stridulate and produce sound. In our study, we maintained silent environments by excising a small wing structure necessary for sound production. Thus, in our study, while we were still able to quantify stridulatory bouts by observing the stereotypical wing movements associated with stridulation (see also Logue et al 2010), the male crickets were unable to produce sound.

The increased level of aggressiveness associated with the lack of acoustic input found in our study has interesting implications for field populations. In the field, flatwing males are more responsive to playbacks of calling song, suggesting that they may act as satellites to the few remaining calling males (Zuk et al 2006). Adding strength to this finding, laboratory-reared males of both morphs exposed to silent environments were more responsive to playbacks than males from song-dense environments (Bailey et al 2010). As a consequence, males may not only be more likely to come into contact with females (Bailey et al 2010), but also conspecific rivals as multiple males attempt to take up position near a calling individual, increasing the likelihood of aggressive encounters. Further, male song functions in preventing the escalation of a battle. Flatwing and artificially-silenced males lack the ability to produce song, and aggressive encounters involving these males are characterized by greater levels of aggression than encounters between calling males (Logue et al 2010). Our results suggest that above and beyond morph, the acoustic environment experienced by males influences levels of aggression, and that encounters in the field between males in silent environments should be characterized by higher than normal levels of aggression, though this remains to be tested.

While our measures of behavioral consistency are cruder than those utilized in Kortet and Hedrick (2007), we found results similar to theirs in that male crickets that were the first to begin moving after the removal of the divider were also more likely to initiate an aggressive interaction, as well as to win agonistic encounters. To our knowledge, this is the first demonstration of a potential behavioral syndrome in our study species. Interestingly, we did not detect any influence of social experience on the expression of a behavioral syndrome. That is, acoustic environment did not alter the relationship between being first to move and initiating an aggressive interaction in male crickets. This is interesting because Sih et al (2004) identified social experience as a potentially powerful driver of behavioral syndromes by either setting the stage for a syndrome or breaking up an existing syndrome, and recent work indicates that social experience influences the expression of behavioral syndromes in at least some species (Lihoreau et al 2009, Aragón 2011, Sih 2011, though see Niemelä et al 2012). However, because we only used males in our study, our interpretation of the role of social experience in mediating behavioral syndromes in *T. oceanicus* is somewhat limited. While several studies investigate the presence of behavioral syndromes within a single sex (Hedrick 2000, Kortet and Hedrick 2007, Lihoreau et al 2009), sex differences in behavioral correlations exist (Aragón 2011), and these may generate dramatically different evolutionary consequences (Pruitt and Riechert 2009). Female crickets are generally considered less aggressive than males, though female aggression towards males and other females exists in this species (Fuentes and Shaw 1986; personal observation) and in *G. campestris* (Rillich et al 2009). In *G. campestris*, females only displayed aggressive behavior towards other females in the presence of male song (Rillich et al 2009), indicating a possible role for social experience to mediate aggression and other behaviors in females.

Age did not affect the relationship between first movement and initiation of aggressive encounters. In both experiments, males that began moving about the arena first were more likely to initiate aggressive encounters, despite the age differences in the two experiments. This contrasts with the previous work on male crickets, in which individual aggression tended to increase with age in both *G. bimaculatus* (Nagamoto et al 2005) and *G. integer* (DiRienzo et al 2012). However, two important differences between our study and other work should be noted. One, we did not measure the amount of summed aggression in across-environment interactions (experiment 2). Rather, we noted which individual initiated and won each encounter, so we were unable to detect changes in overall aggression as a function of age. Second, the crickets used in experiment 1 were 24-26 days post-adult eclosion, while those used in DiRienzo et al (2012) were 20-40 days post-adult eclosion. Thus, the ages of our crickets would be in the lower range of those used in DiRienzo et al (2012). In *G. bimaculatus*, age did not influence aggression in individuals reared at high density, but for individuals reared in isolation, aggressiveness increased with age, with crickets seven days post-eclosion displaying more aggression than males one day post-eclosion (Nagamoto et al 2005). Our study also contrasts with previous work in that we found that social experience did not influence which cricket won aggressive encounters in across-environment interactions. In *G. integer,* males reared in silence were more likely to win encounters (DiRienzo et al 2012), but in our study, males from song and no song environments were equally likely to win or lose encounters.

Finally, our results underscore the fact that evolutionary change can generate a cascade of events affecting populations. In guppies, adaptation to local predation regimes has marked ecological consequences (Bassar et al 2010), and in many insects, the evolution of alternative mating strategies generates situations facilitating sperm competition, which can in turn, exert its own influence on populations (Simmons 2001). The evolutionary loss of a sexual signal in our study species alters the social environment experienced by all individuals, which then alters the expression of a behavioral trait, aggression, likely by altering perceived population density (see also Bailey et al 2010).

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Table 2.1. Description of the behaviors scored for each aggression trial.

Behavior	Description
stridulation	Bouts of wing rubbing; one bout consisted of raising the wings, rubbing them together, then lowering the wings (see Logue et al 2010)
aggressive act	any of the following actions: antennal lashing, kicking, biting, chasing, juddering, mandible flaring or grappling
initiation	beginning an aggressive encounter, usually by approaching and making contact with another male
retreat	movement away from an opponent after aggressive acts or active avoidance of an opponent

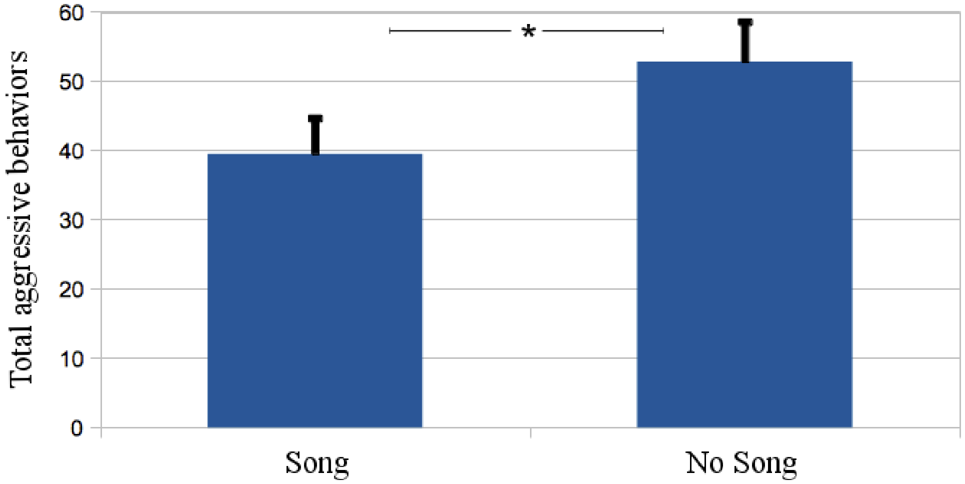

Figure 2.1. Mean summed aggressive behaviors for male cricket pairs from song and no-song environments.

Chapter 3

Multimodal signal compensation: Do field crickets shift sexual signaling modality after the loss of acoustic communication?

Brian Gray[1], Nathan W. Bailey[2], Michelle Poon[1], Marlene Zuk[1,3]

1. Department of Biology, University of California, Riverside, USA
2. Centre for Biological Diversity, University of St Andrews, UK
3. Department of Ecology, Evolution, and Behavior, University of Minnesota, USA

ABSTRACT

Several hypotheses could explain the evolution of multi-modal signals. One possibility is that such signals allow for communication even when one signalling modality is temporarily unavailable. However, little is known about the consequences of the permanent, evolutionary loss of a signal modality. We used the field cricket *Teleogryllus oceanicus* to test the hypothesis that the loss of one mode of signaling can be accommodated by flexibly switching to another, pre-existing modality. Field crickets employ cues that carry social information both in the form of long-range acoustic signals and short-range cuticular hydrocarbons (CHCs), but males in some *T. oceanicus* populations have permanently lost the ability to sing due to a morphological mutation erasing sound-producing structures on their wings. In assays testing responsiveness to substrate-borne CHCs, *T. oceanicus* female crickets responded to the presence of male, but not female, CHCs, which is consistent with known sexual dimorphism in field cricket chemical cues. However, we found no evidence for signal compensation in male crickets that have experienced an evolutionary loss of acoustic signals: females did not differentially respond to the CHCs of constitutively silent males compared to those of normal males. The ability of organisms to adaptively shift from one signaling modality to another following the evolutionary loss of a signal is likely to be constrained by both the degree to which signal production and receiving is flexible, plus the existence of suitably pre-adapted alternative modalities.

INTRODUCTION

In recent years, several non-exclusive hypotheses have been developed to explain the evolution of complex signals comprised of multimodal or multicomponent signals (Partan and Marler 1999, Candolin 2003, Hebets and Papaj 2005, Partan and Marler 2005). For example, the 'multiple messages hypothesis' maintains that a complex signal may be comprised of several units that each convey different information about a sender, while the 'redundant signal hypothesis' posits that different signals each convey the same information and exist to safeguard against imperfect signal coding (Hebets and Papaj 2005).

Another hypothesis, the 'efficacy backup hypothesis', maintains that varying environmental conditions can hinder the transmission and reception of a signal, so signalers may utilize multiple signal modalities to increase the likelihood of successful communication (Hebets and Papaj 2005). That is, multiple modalities may allow the propagation and reception of a signal even if one or more modalities are blocked (Candolin 2003, Partan 2004). Support for this hypothesis comes from several empirical avenues. Male satin bowerbirds, *Ptilonorhynchus violaceus,* actively enhance their bowers by adding sticks and reducing latency to paint their bowers when bower decorations are experimentally removed (Bravery and Goldizen 2007). Organisms living in temporally, spatially or otherwise heterogeneous environments also demonstrate compensatory mechanisms. For example, male Alpine newts, *Mesotriton alpestris*, use relatively more olfactory cues when courting in the dark, and more visual cues when courting in brighter conditions (Denoël and Doellen 2010). Male wolf spiders, *Schizocosa ocreata,* which normally produce substrate-borne courtship signals, perform more visual courtship signals when on surfaces that preclude the transmission of substrate-borne vibrations (Gordon and Uetz 2011). Males of this species even compensate for the loss of one or both forelegs by performing a particular courtship display at a higher rate than males with both forelegs (Taylor et al 2006).

The individual signalling components of complex signals might be used to provide a layer of redundancy, enabling organisms to flexibly match signal or cue modality to the prevailing environmental conditions. However, few studies have examined how organisms respond to the *permanent* evolutionary loss of a sexual signal, rather than the loss of signal or cue production or transmission under varying environmental conditions (though see Taylor et al 2006). Given that varying environmental conditions may induce organisms to shift investment between two or more sexually selected traits (Basolo 1998), we hypothesized that signaling individuals compensate for the evolutionary loss of a sexual signal by increasing the use of other, pre-existing cues. To test this, we capitalized on a field cricket system in which a primary signaling modality has been evolutionarily lost on a contemporary timescale.

The field cricket *Teleogryllus oceanicus* is widely distributed from northern and western Australia to Hawaii and throughout the Pacific (Otte and Alexander 1983, Otte 1994). Males of this species use at least two signal modalities in mate attraction: a long-range acoustic signal, calling song (Balakrishnan and Pollack 1996, Zuk and Simmons 1997, Rebar et al 2009); and a sexually dimorphic contact (short-range) chemosensory cue in the form of cuticular hydrocarbons (hereafter *CHC*s; Tregenza and Wedell 1997, Thomas and Simmons 2008a). Females also produce CHCs, but unlike some other insect species that produce airborne or otherwise volatile cues, chemical communication appear to be limited to contact chemoreception in crickets, including *T. oceanicus* (Thomas and Simmons 2008b). In two Hawaiian populations of *T. oceanicus*, some males have lost the ability to produce song. A recent wing mutation, *flatwing*, renders males on the Hawaiian Islands of Kauai and, more recently, Oahu, obligately silent (Zuk et al 2006, Tinghitella 2008). Flatwing males represent >90% of the males on Kauai, and approximately 50% of all males on Oahu (M. Zuk, unpublished data).

Cuticular hydrocarbons are found on insect exoskeletons. They are thought to have evolved to resist desiccation, but have evolved into a chemical form of communication in many insects (Blomquist et al 1998). In *T. oceanicus*, cuticular hydrocarbon expression is heritable (Thomas and Simmons 2008b) and subject to sexual selection (Thomas and Simmons 2009a, Thomas and Simmons 2010). However, CHC expression in this species is also highly plastic. Male normal-winged crickets reared in silent environments, such as one that would be experienced in a low-density population or in a population consisting mainly of flatwing males, increased the relative expression of CHCs that females find attractive (Thomas et al 2011). Moreover, subordinate males of this species increase the relative expression of the same attractive CHCs (Thomas and Simmons 2009b), and hydrocarbon profiles can change within short time spans (Thomas and Simmons 2011). In addition to being deposited on other crickets during mating and other interactions (Thomas and Simmons 2009c), CHCs can be deposited on the substrate (Kortet and Hedrick 2005, Bailey 2011). These CHCs remain detectable for long periods of time, having been successfully identified from museum specimens (Brenner et al 2003). As such, substrate-borne CHCs represent a stable source of information that conspecific crickets may access and utilize during everyday interactions and may inform behavioural decision-making processes (Bailey 2011).

Given existing phenotypic plasticity and the known role of CHCs in mediating mate choice (Kortet and Hedrick 2005, Thomas and Simmons 2008), we hypothesized that flatwing males compensate for their inability to produce a sexual signal by upregulating the production of attractive chemical cues. To test this, we utilized a female response assay, measuring how much time female *T. oceanicus* spent in association with substrate-borne CHCs from each male morph. We also measured female response to CHCs from other females to control for a generalized response to any chemosensory cues. In this study, we used crickets from Oahu because both male morphs are represented in roughly equal numbers (M. Zuk, unpublished data), so females are likely to encounter both morphs under natural conditions.

We predicted that if CHCs can function as a backup to acoustic sexual signals, female crickets would spend more time in association with CHCs from normal-winged males, but not spend more time in association with CHCs from conspecific females than expected by chance. Further, we predicted that flatwing males compensate for a lack of sound production by producing more attractive CHCs, in which case females would spend more time attending substrate-borne CHCs from flatwing males than they would with those produced by normal-winged conspecifics.

METHODS

We used a female response assay to determine the attractiveness of CHCs from different crickets. Females were allowed to interact with substrate-borne CHCs from conspecific females, flatwing males, or normal-winged males and could choose to spend time on substrates with these CHCs or on substrates free of CHCs. Two distinct groups of crickets were reared. One group, consisting solely of females, was reared for use in female-response assays ('focal females'). The other group, consisting of females, flatwing males, and normal-winged males, provided crickets for CHC deposition in the testing arenas ('CHC donor crickets'). Crickets in this latter group were reared individually.

General rearing

Crickets were taken from a large, outbred population originating from Oahu, Hawaii and supplemented with the offspring of 12-25 field-caught females approximately once annually. Crickets were selected as late-instar juveniles as soon as sex differences were apparent. Females for behavioral trials were removed to female-only 1.8L containers with cardboard egg crates as shelter. No more than six females were placed into a container. Male and female CHC donor crickets were placed individually into 118ml containers with a piece of cardboard egg crate as shelter. All crickets were given Purina rabbit chow and water *ad libitum* and kept on a 12:12 hour light:dark cycle at 25˚C in a controlled temperature growth chamber. Food and water were replaced twice weekly and all individuals were checked at least five times per week for newly emerged adults. At the end of the study, crickets were either placed back into the general population or euthanized by freezing.

Test arena

All trials were performed in circular test arenas measuring 1m in diameter and 30 cm deep (figure 1). Arenas were constructed by adhering aluminum flashing to the edge of a circular styrofoam base plate. Arenas contained a central starting area, covered by an inverted 118ml plastic cup attached to a pulley system. This system allowed the cup to be remotely removed by the experimenter without disturbing the cricket being used in the trial.

CHC deposition

The bottom of the test arena was covered with a layer of brown packing paper, lightly moistened to ensure conformation to the dimensions of the arena. The paper was divided into four sections of equal area: two for CHC deposition and two CHC-free control areas (Figure 1). Cricket chemical cues were deposited by lightly rubbing the surfaces of a single donor cricket against the paper in the CHC-deposition regions until the two regions were completely covered. Control areas were sham-treated by rubbing with a clean watch-glass. Females have been shown to detect and respond to chemical cues deposited on paper substrates in this and similar fashions (Kortet and Hedrick 2005, Bailey 2011).

Donor crickets used in this study were 4-26 days of age post-adult eclosion; in a pilot study, we tested whether female responses varied depending on the age of individuals used to deposit chemosensory cues. Donor cricket age did not influence female behavior in the pilot trials (see Results), so donors of all age classes were pooled for subsequent trials. A different donor cricket was used in each trial.

Female Association Trials

All trials were conducted at 25 +/-1.5˚C in a dark room equipped with red light for viewing. Intact female crickets possessing both antennae were randomly assigned to treatments (experiencing CHCs from conspecific females, normal-winged males, or flatwing males), and a single female (hereafter *focal female*; 6-10 days post-adult eclosion) was placed into the starting area under an inverted plastic cup and allowed to acclimate for two minutes. The inverted cup spanned the CHC-deposition and control areas, enabling the focal female to interact with donor cricket CHCs during the acclimation period. After the acclimation period, the inverted cup was lifted and timing began. We recorded the female's latency to move, as well as the amount of time she spent in CHC and control areas. Each trial lasted five minutes.

Statistical Analysis

Data were non-normal and transformations failed to achieve normality, so we utilized nonparametric analyses. We used Wilcoxon tests to determine whether females spent more time in association with substrate-borne chemical cues than would be expected by chance. Because we made multiple comparisons, we used a Bonferroni-corrected α of 0.017.

We were also interested in the factors that influenced how long females spent in areas with chemosensory cues. Some females did not move immediately upon the start of each trial, so we recorded the time elapsed movement commenced and subtracted this from the total trial time to obtain the time spent moving. The amount of time a female spent in association with the CHC-deposition areas of the arena was divided by her time spent moving (see Kortet and Hedrick 2005). Kruskal-Wallis tests were used to compare the latencies to move and the proportion of time females spent in association with each of the three donor cricket types. Post-hoc comparisons, when appropriate, were performed using Wilcoxon tests.

We also tested for correlations between focal female and CHC donor body mass and time spent in association with CHC-saturated areas using Spearman's ρ. All analyses were performed in JMP 10.0 (SAS Institute, Cary, NC, USA).

RESULTS

In our pilot study, the age of the chemical cue donor crickets did not affect female latency to respond to substrate-borne chemical cues (Kruskal-Wallis $\chi 2 = 1.964$, df = 2, N = 180, p = 0.375) or the percentage of time spent in association with chemical cues (Kruskal-Wallis $\chi 2 = 1.853$, df = 2, N = 180, p = 0.396).

Female movement latency

Female movement latency at the start of trials differed depending on the type of CHC that had been previously deposited on the substrate (Figure 2). Females took longer to begin moving when CHCs were deposited by flatwing males, compared to those presented with CHCs from normal-winged males or females (Figure 2; Kruskal-Wallis $\chi 2 = 10.172$, df =2, N = 270, p = 0.006).

Female associations with substrate-borne CHC cues

Females spent more time in association with CHCs from normal-winged and flatwing males than expected by chance, but did not spend more time in association with CHCs from females than expected by chance (Table 1, Figure 3).

The identity of the donor cricket affected female responses to substrate-borne CHCs (Kruskal-Wallis; $\chi 2 = 21.471$, df = 2, N = 270, $p < 0.001$). Females spent less time in association with chemosensory cues from females than with either flatwing or normal-winged males (figure 3), but their time spent with CHCs from flatwing and normal males did not differ (Wilcoxon multiple comparisons; Z = 0.860, p = 0.390).

Female mass had a weak and non-significant effect on the percentage of time spent in CHC-deposition areas (Spearman $\rho = 0.114$, N = 270, p = 0.063), with heavier females tending to spend more time in CHC-deposition regions than lighter females. The mass of CHC donor crickets did not affect the percentage of time spent by focal females in regions with substrate-borne CHCs (Spearman $\rho = -0.02$, N = 270, p = 0.743).

DISCUSSION

While sexual signal loss is more common than previously thought (Wiens 2001), the downstream effects that such losses have on the ability of organisms to locate and evaluate potential mates are poorly understood because we rarely observe such loss happening on a contemporary timescale (Svensson and Gosden 2007). Using female response assays, we were able to test the potential role of CHCs in mate location in a population of field crickets that have experienced the evolutionary loss of a dominant sexual signaling modality: acoustic songs. Females spent more time in association with substrate-borne CHCs from both types of male than with cues from females, a finding consistent with observations of sex-specific CHC profiles in other species of crickets (Leonard and Hedrick 2009) and adding to a number of existing studies that indicate that females utilize chemosensory cues in mate choice (Ivy et al 2005, Kortet and Hedrick 2005, Weddle et al 2013).

Our results are consistent with what is known about female response to the different male morphs in this species (Bailey et al 2008). Typically, males produce a long-range calling song to attract females (Balakrishnan and Pollack 1996). Upon contact with a female conspecific, males produce a second song, known as courtship song. This song is thought to function in releasing female mounting behavior (Balakrishnan and Pollack 1996), and is used by females in mate choice (Rebar et al 2009). As such, flatwing males should be at a double disadvantage in securing mating opportunities in that they are neither able to attract females nor elicit female mounting. Indeed, female crickets from across the range of *T. oceanicus* showed reduced mounting rates when presented with flatwing males from Kauai (Tinghitella and Zuk 2009). However, this study did not measure female mating rates with flatwing males from Oahu, which are used in the current study, nor compare female mounting rates of normal and flatwing males taken from a single population. In a separate study, females from Kauai were as likely to mount a flatwing male as an artificially-silenced normal-winged male regardless of the presence of courtship song (Bailey et al 2008). Further, female latency to mount and accept a spermatophore did not differ with respect to male morph (Bailey et al 2008), suggesting that beyond the ability to sing, flatwing males do not differ from normal-winged males from the same population in eliciting female mounting and mate acceptance.

Contrary to our predictions, females did not preferentially associate with CHCs from flatwing males. However, context may play an important role in compensation. For example, wolf spiders utilise multimodal courtship displays, with some species switching to displays that best suit the transmission environment (i.e visual displays in the light or when on substrates that attenuate vibrational signals; Gordon and Uetz 2011). In this case, environmental heterogeneity may select for complex signals. In crickets, however, sound production is the primary and dominant form of sexual signaling, and the only long-range modality available to males. Further, most crickets do not live in environments where acoustic signals would be rapidly attenuated (though see Masaki and Shimizu 1995), so there may not be selection for the use of multimodal signals in mate location, at least not in the context of the efficacy backup hypothesis. Further, this hypothesis generally assumes phenotypic covariance between the two communication modalities, much as the redundant signals hypothesis does. Because flatwings cannot produce song, we were unable to measure any relationship between acoustic and chemical cues. However, Leonard and Hedrick (2010) found no support for the redundant signals hypothesis in the field cricket *Gryllus integer,* though they did find evidence that acoustic signals and chemical cues function in a manner consistent with the multiple messages hypothesis, which predicts that the two modalities will vary independently of one another. Male *G. integer* that produced attractive calling song did not necessarily produce attractive chemical cues (Leonard and Hedrick 2010). In *T. oceanicus*, chemical cues reduce the time it takes for females to respond to acoustic signals, but only when both modalities are presented simultaneously (Bailey 2011), suggesting that the multimodal signals in this species serve in a multiple messages context, each providing receivers with different information about the signaler. Moreover, recent work indicates that courtship song and CHCs function in the context of multiple messages in this species (Simmons et al, in press).

The lack of consistency between acoustic signals and chemical cues aligns well with the known plasticity in cuticular hydrocarbon expression in *T. oceanicus*. Males can rapidly alter hydrocarbon expression in response to social status, and dominant males have distinctly different hydrocarbon profiles than subordinate males (Thomas and Simmons 2009b, 2011). Further, normal-winged males of this species have been found to increase the production of attractive hydrocarbons when reared in silent environments (Thomas et al 2011). Instead of morph-specific changes in hydrocarbon expression, male crickets may be highly sensitive to changes in acoustic environment, regardless of morph.

Because the presence of flatwings in a population alters the acoustic landscape experienced by all crickets, males may respond to this altered acoustic environment. For example, both flatwing and normal-winged males increase immunity when exposed to song (Bailey et al 2011). Thomas et al (2011) assayed the CHC profiles of normal-winged males reared in either the presence or absence of song, but finer scale adjustments in male response and production of hydrocarbons might be possible. Rearing male *T. commodus* in environments that vary in song quality affects adult body size (Kasumovic et al 2011), while varying call density affects both adult reproductive effort (Kasumovic et al 2011) and lifespan (Kasumovic et al 2012). Thus, variation in exposure to song influences adult behavior and physiology, and as such, may also affect hydrocarbon expression. In this case, inter-individual differences in male CHC expression may overcome any differences in CHC expression between normal-winged and flatwing males.

Alternatively, we may not have detected compensation because there may not have been time enough for compensation to have evolved. In addition to being highly plastic, CHCs are subject to sexual selection, and females display preferences for males that have CHC profiles that differ from the population average (Simmons et al, in press). In this form, selection is disruptive, favoring males with CHC profiles at extremes, though stabilizing selection has also been detected for some components of male *T.* oceanicus CHC profiles (Thomas and Simmons 2009a). Females also prefer males with CHC profiles dissimilar to their own (Thomas and Simmons 2011). Given this, it is entirely possible that selection has yet to act in favoring compensation for the loss of acoustic signals by chemical cues.

We found no evidence that crickets increase their use of contact chemosensory cues after the evolutionary loss of a long-range acoustic signal. Adaptive shifts from one signal modality to another may be constrained by a number of factors. Several hypotheses exist to explain the origin of multimodal communication, and some of these may facilitate shifts between modalities more easily than others. While shifts may be more common in organisms that experience temporary signal loss in heterogeneous environments, such shifts may not occur following the permanent, evolutionary loss of a signal modality. The ability of organisms to flexibly shift between modalities may well depend on the availability of other suitable, pre-existing modalities to convey information, as well as the presence of appropriate receivers, and enough time for selection to facilitate shifts between modalities.

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Table 3.1. The percentage of time females spent in areas with CHC deposition, and whether that differed from expectations based on chance for CHCs from female, normal-winged males, and flatwing males. All three are presented separately.

Donor cricket	N	% time females spent in CHC-deposition areas	Z†	P	R (Z/sqrtN)	Differs from chance?
Female	90	52.7	388.5	0.112	40.96	No
Normal-winged male	90	63.8	1626	**<0.001**	172.5	Yes
Flatwing male	90	61.4	1269	**<0.001**	133.7	Yes

†Wilcoxon signed-ranks test. Significant p-values are indicated in bold; Bonferroni-corrected α of 0.017.

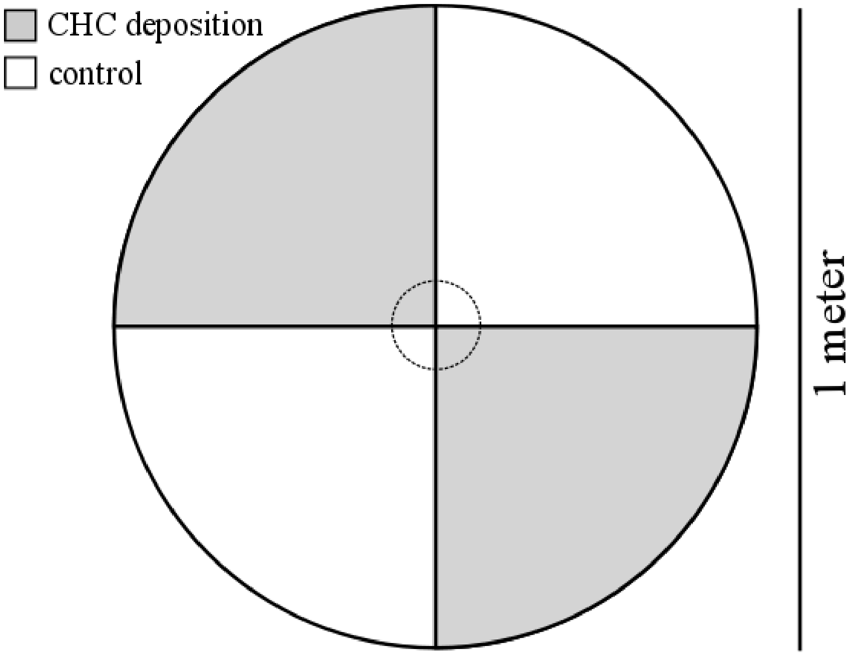

Figure 3.1 Diagram of the testing arena as viewed from above. Female crickets began each trial in the center of the inner circle (denoted by a dashed line).

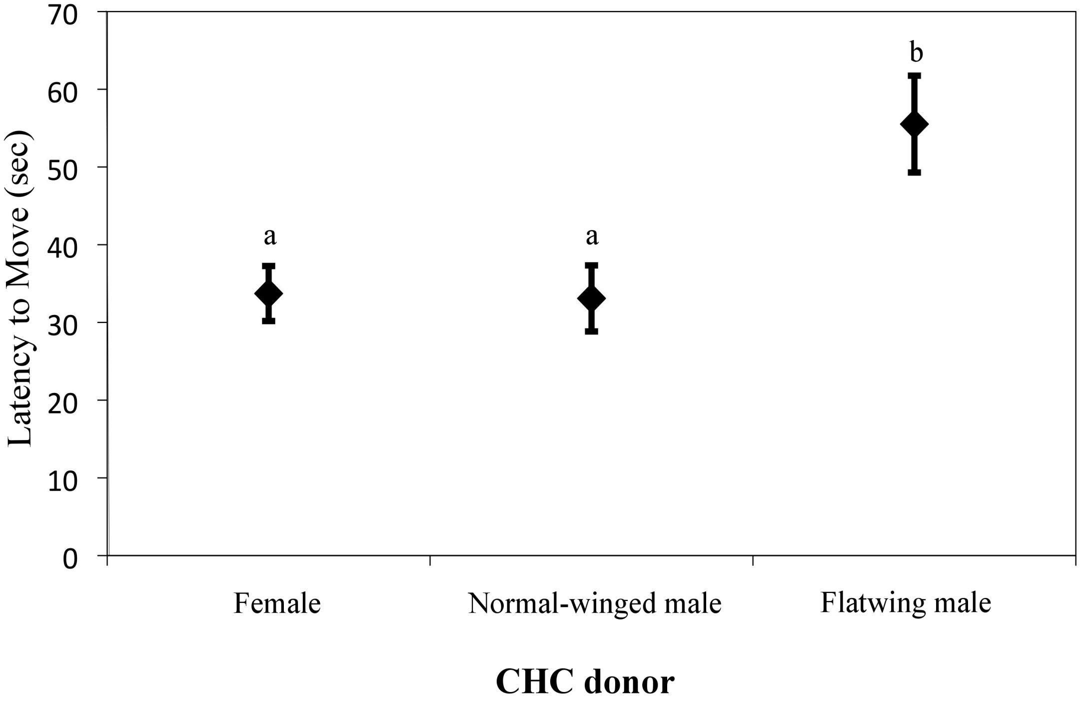

Figure 3.2 Mean female latency to move for each treatment. Bars represent one standard error. Treatments sharing the same letter are not significantly different from one another.

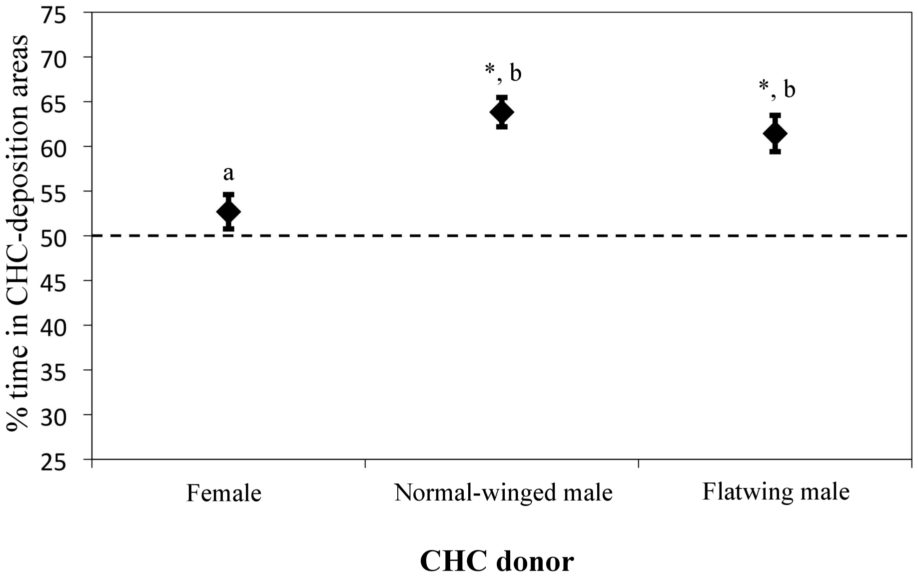

Figure 3.3 Mean percentage of time females spent in CHC-deposition areas. Bars represent one standard error. The null line is displayed (50%). Treatments sharing the same letter are not significantly different from one another. Asterisks denote that a treatment differs significantly from chance (50%).

Chapter 4

Does the loss of an acoustic signal affect the age structure of a parasitized population of field crickets?

Brian Gray[1] and Marlene Zuk[2]

1. Department of Biology, University of California, Riverside, 900 University Avenue, Riverside, CA 92521 USA
2. Department of Ecology, Evolution, and Behavior, University of Minnesota, 1987 Upper Buford Circle, Saint Paul, MN 55108 USA

ABSTRACT

Hawaiian populations of the field cricket *Teleogryllus oceanicus* are subject to an acoustically-orienting parasitoid fly, *Ormia ochracea.* Because only male *T. oceanicus* call, they are considerably more susceptible to lethal parasitism from the fly. However, a novel male morph, *flatwing*, has recently emerged in two populations. This morph lacks the wing structures necessary to produce sound. While this makes mate attraction difficult, it does afford protection against parasitism by precluding acoustic location by the fly. In a previous survey, males from parasitized populations were younger than males from unparasitized populations, likely as a result of parasite-induced mortality. Here, we investigate whether flatwing males are older than their normal-winged congeners due to their ability to escape detection by the parasite. Contrary to our predictions, we did not find flatwing males to be older than normal-winged males, perhaps due to a lack of statistical power to resolve differences between groups. However, the average ages of flatwing males, normal-winged males, and females in our survey are consistent with the ages of normal-winged males and females from parasitized populations in a previous study.

INTRODUCTION

Contemporary evolution is more common than previously thought, often occurring in response to heterogeneous or changing environments (Hendry and Kinnison 1999, Reznick and Ghalambor 2001, Carroll et al 2007). Importantly, Carroll et al (2007) note that evolutionary change can have a number of consequences, including affecting local ecological processes as well as altering demographic parameters. For example, Trinidadian guppies *Poecilia reticulata* from environments where predation is biased toward adult age classes rapidly adapt to environments where predation is focused on juveniles when introduced, maturing at a later age and attaining a greater body size at maturity (Reznick et al 1990). In another study, guppies adapted to low-predation environments affected common-garden mesocosms differently from guppies from high-predation environments (Bassar et al 2010). Low-predation guppies consumed more algae and fewer invertebrates, which can alter ecosystem processes (Bassar et al 2010). However, contemporary evolution need not be limited to the appearance of novel traits, and may instead involve trait loss.

We took advantage of one such episode of contemporary evolution – the loss of a sexual signal, male calling song – in a cricket to measure the effects of evolution on a demographic parameter, age structure. The Pacific field cricket, *Teleogryllus oceanicus*, is widely distributed in Australia and the Pacific Islands (Otte and Alexander 1983, Otte 1984), and was introduced to Hawaii by 1877 (Kevan 1990). *T. oceanicus* breeds continuously throughout the year, with approximately 3-4 generations per year (Tinghitella and Zuk 2009). In Hawaii, *T. oceanicus* co-occurs with an acoustically-orienting, North American parasitoid fly, *Ormia ochracea* (Zuk et al 2006, Lehmann 2003). Male *T. oceanicus* call to attract females, but calling also attracts gravid female flies that deposit larvae on and around calling males. The larvae enter the cricket's body cavity, where they consume host tissue until their emergence 7-10 days later, killing the host in the process (Adamo et al 1995). Calling, therefore, necessitates a balance between natural and sexual selection. Natural selection favors less or no calling to avoid parasitism, but sexual selection favors males who call frequently, as these males attract mating partners (Zuk et al 1998).

In addition to influencing host physiology, morphology, and behavior in *T. oceanicus* (Rotenberry et al 1996, Zuk et al 1998, 2006), *O. ochracea* also impacts demographic parameters. Male *T. oceanicus* from parasitized populations are, on average, younger than males from unparasitized populations, likely owing to parasite-induced mortality (Simmons and Zuk 1994). Perhaps the most dramatic example of a host response to *O. ochracea* is a wing mutation, *flatwing,* that occurs in Hawaiian populations on Kauai and Oahu (Zuk et al 2006). This single-locus, sex-limited mutation alters male wing structure, precluding sound production in males bearing the trait (Tinghitella 2008), and represents one of the fastest cases of contemporary evolution documented in a wild population (Zuk et al 2006). While flatwing males encounter difficulties in mate attraction because of their inability to produce the songs typically used in this endeavor, these males are protected from parasitism from *O. ochracea* (Zuk et al. 2006, unpublished data).

Here we investigate how this loss of calling ability has affected the age structure in the field. Specifically, if *O. ochracea* increases mortality in normal-winged males (Simmons and Zuk 1994), then flatwing males, being freed from *Ormia*-induced mortality, should be, on average, older than normal-winged males. Previous work on parasitized populations of this species found no difference in the mean ages of female and normal-winged males (Simmons and Zuk 1994). Accordingly, we predicted that females and normal-winged males would not differ in age. Age determination of insects can be accurately performed by counting the number of cuticular layers in sections of body parts (Neville 1963a, b). These growth layers are deposited daily, and are also influenced by circadian rhythm and photoperiod (Neville 1965). This technique has been refined for use with wild-caught crickets (Zuk 1987) and has been used to determine the age structure of various cricket populations (Murray and Cade 1995), including *T. oceanicus* (Simmons and Zuk 1994).

METHODS

Accuracy of the Aging Method

For calibration, legs were taken from crickets of known age reared at 25ºC in the laboratory on a 12h:12h light:dark cycle, then frozen at -20ºC until sectioning (see below). Counts of growth rings (figure 1) were made without knowledge of the actual age of the individuals. As 30 days is the upper limit for most individuals found in the field (Zuk 1987, Simmons and Zuk 1994, Murray and Cade 1995), we used crickets that ranged in age from 1 - 30 days post-adult eclosion.

Age distribution of field populations

Legs were collected from field-caught individuals on Oahu in August 2010 (N = 90) and February 2012 (N = 95). Legs were kept frozen at -20ºC until sectioning.

Sectioning

Tibial cross-sections, 16-25μm in thickness, were obtained using a cryostat. A minimum of six intact sections were obtained for every individual. Sections were mounted on a glass slide and viewed at 400x magnification using phase-contrast microscopy (Simmons and Zuk 1994). Growth rings were counted for a minimum of three sections per individual, and the maximum number of rings counted in any section was used in data analysis (Murray and Cade 1995). All counts of growth rings were made blind to the morph and sex of the individuals collected, as well as the sampling year.

Data Analysis

Age distribution of field populations

To test for differences among cricket morphs, we used an ANOVA followed by Tukey HSD post-hoc comparisons. We entered cricket morph (female, normal-winged male, or flatwing) and year of sampling as factors, as well as the interaction between the two. Because of low statistical power and because the question of interest was the comparison of mean ages among morphs within each sampling year, we ran one-way ANOVAs followed by Tukey post-hoc comparisons using cricket morph as the main factor for each year of sampling separately. All analyses were performed in JMP 10.0.0 (SAS Institute, Cary, NC, USA). Results of ANOVAs are presented along with effect sizes (partial-eta squared), and data are presented as means ± se.

RESULTS

Accuracy of the aging method

A regression of the number of growth rings present in tibial cross-sections on the actual age of individuals was significant (Figure 1; $R = 0.93$, $p < 0.001$, $n = 21$).

Age distribution of field populations

Both cricket morph ($F_{2,179} = 3.401$, $p = 0.036$, $\eta_p^2 = 0.037$) and the year during which individuals were sampled ($F_{1,179} = 41.966$, $p < 0.001$, $\eta_p^2 = 0.189$) significantly affected the mean age of individuals. The interaction between morph and year was not significant ($F_{2, 179} = .435$, $p = 0.648$, $\eta_p^2 = 0.0005$), indicating that the mean ages of morphs relative to one another did not differ across sampling periods.

Crickets sampled in 2012 were older than those surveyed in 2010 (mean age in 2012 = 9.75 ± 0.251 days, mean age in 2010 = 7.47 days ± 0.252 days; see figure 3 for age distributions). Tukey post-hoc comparisons indicated that females were, on average, younger than flatwing males ($p = 0.036$), but not different in age from normal-winged males ($p = 0.910$). The two male morphs did not differ significantly in age ($p = 0.143$). However, our tests lacked statistical power (see also Simmons and Zuk 1994). To resolve this, and because the question of interest was how age varies among the morphs within sampling periods, we ran separate ANOVAs for each sampling period.

In both 2010 and 2012, age was not affected by cricket morph (2010: $F_{2, 87} = 2.009$, $p = 0.140$, $\eta_p^2 = 0.044$; 2012: $F_{2, 92} = 2.102$, $p = 0.128$, $\eta_p^2 = 0.044$). We report effect sizes for each pairwise comparison using Cohen's d (Nakagawa and Cuthill 2007; see tables 1a and 1b).

DISCUSSION

While sexual signal loss appears to be a fairly common occurrence (Wiens 2001), we often do not have the opportunity to measure its potential consequences because it rarely is observed on a contemporary timescale (Svennson and Gosden 2007). Here, we measured the age structure of a population of field crickets that has evolutionarily lost a sexual signal, a signal exploited by a lethal parasitoid. Contrary to our predictions, there were no significant difference in the mean ages of flatwing and normal-winged males. While we found that flatwing males tend to be older than females, the differences between females and flatwing males disappeared when we analyzed the data separately for each sampling period. Our results are generally consistent with previous work on this species. The ages of crickets sampled in our study are similar to those assayed in Simmons and Zuk (1994), who also found no difference in the mean ages of female and normal-winged males from a parasitized Hawaiian population.

Interestingly, females in unparasitized populations of *T. oceanicus* were younger on average than normal-winged males (Simmons and Zuk 1994), which runs counter to the idea that calling males should be younger than females due to parasite-induced mortality. Murray and Cade (1995) found similar results in a comparison of three species of *Gryllus* that differed in parasitism rates. In two species not subject to parasitism from *Ormia*, females were either younger than males or did not differ from males in mean age. But in *G. integer,* males are parasitized by *O. ochracea,* and are younger than females (Murray and Cade 1995). Simmons and Zuk (1994) argue that females experience increased mortality rates during phonotaxis, when they actively move toward calling males, possibly due to predation. The differences in age between males and females disappeared in a parasitized population from Hawaii (Simmons and Zuk 1994). While females from parasitized populations did not differ in mean age from females from unparasitized populations, males in parasitized populations were younger, presumably because of *Ormia*-induced mortality, than males in unparasitized populations (Simmons and Zuk 1994).

Given this, it is notable that flatwings did not appear to be older than normal-winged males in our study despite not being subject to parasitism from *O. ochracea*. While flatwings are inherently protected from acoustically-orienting parasitoids, normal-winged males are not defenseless. Crickets can utilize a variety of behavioral defenses to avoid parasitism, from shifting peak calling activity to times when parasitoids are less active (Zuk et al 1993, Bertram et al 2004), being more sensitive to disturbances when calling (Lewkiewicz and Zuk 2004), or grooming to remove parasitoid larvae before infestation (Vincent and Bertram 2010). Both male morphs can utilize these behavioral defenses, potentially eroding differences in mean age between the morphs as normal-winged individuals escape lethal parasitism. Further, both male morphs may act as 'satellites', remaining in relatively close proximity to calling males and attempting to intercept responding females (Zuk et al 1993, Zuk et al 1995, Zuk et al 2006, Bailey et al 2010). While this frees them from parasitoid pressure (Zuk et al 2006), the increased movement associated with satellite behavior, including phonotaxis toward calling males, may make satellite males more susceptible to predation or other risks of mortality and reducing the mean age of satellite males in the population.

Age structure has potentially important implications for the maintenance of multiple male morphologies within a population. While multiple male morphologies may be maintained via negative frequency-dependent selection (Sinervo and Lively 1996), Darwin argued that for multiple male morphs to co-exist in a population, the two morphs must have equal lifetime fitness (Darwin 1874, Shuster and Wade 2003). Further, the novel phenotype must have greater fitness than that of the established phenotype in order to become established in a population (Shuster and Wade 2003). As such, flatwing males must experience some gain in fitness relative to normal-winged males in order to become established in the populations, and should have a lifetime reproductive success equal to that of normal-winged males. While we predicted that flatwing males might live longer than normal-winged males, allowing them additional time to secure mating opportunities, we did not find evidence to support such a prediction. How then are flatwings maintained within the population? One hypothesis may be that they have a longer 'operational reproductive life' (Tsubaki et al 1997), rather than an longer lifespan in absolute terms. One might expect parasitized individuals to invest more in immediate reproduction because their residual reproductive value decreases upon being parasitized, known as the reproductive compensation hypothesis (Minchella and Loverde 1981, Agnew et al 2000). However, crickets infested by *O. ochracea* do not increase reproductive investment (reviewed in Vincent and Bertram 2010). Rather, male crickets decrease reproductive investment, a pattern seen in both *G. texensis* (Orozco and Bertram 2004) and in *T. oceanicus* (Kolluru et al 2002). Some species of crickets, such as *G. bimaculatus, G. rubens*, and *G. integer*, are able to copulate for a few days post-infestation (Adamo et al 1995), and in *G. integer*, calling duration declines gradually after infestation (Cade 1984). However, individuals of other cricket species show diminished reproductive output almost immediately. In *T. oceanicus*, male reproductive output begins to decline rapidly, beginning during the initial stage of parasitoid infestation (Kolluru et al 2002). Thus, parasitized males experience considerable reductions in fitness almost immediately post-infestation, but flatwings, being generally free from parasitism, might continue to reproduce for their entire adult lifespan.

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Table 4.1a Standardized and unstandardized effect sizes for individuals sampled in 2010 (N = 90).

Difference	Mean difference (days)	Cohen's d	95% CI of effect size
Normal male - female	0.486	0.149	-0.108 – 0.408
Flatwing male - female	1.395	0.514	0.280 – 0.747
Flatwing male – normal male	0.909	0.319	0.046 – 0.592

Table 4.1b Standardized and unstandardized effect sizes for individuals sampled in 2012 (N = 95).

Difference	Mean difference (days)	Cohen's d	95% CI of effect size
Normal male - female	0.129	0.084	0.017 – 0.150
Flatwing male - female	0.710	0.457	0.196 – 0.719
Flatwing male – normal male	0.839	0.457	0.195 – 0.718

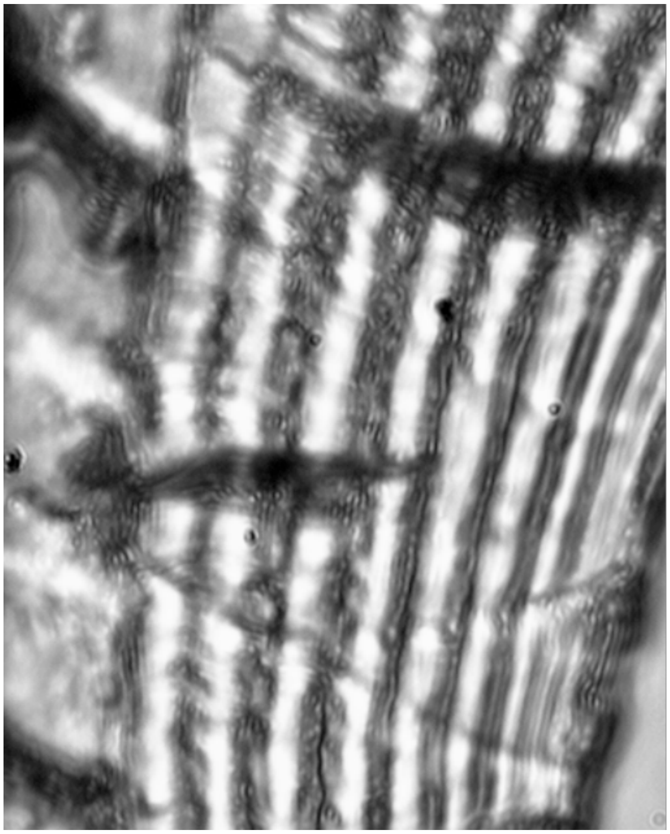

Figure 4.1 Sample photograph of a tibial cross-section showing cuticular growth layers, viewed at 400x magnification.

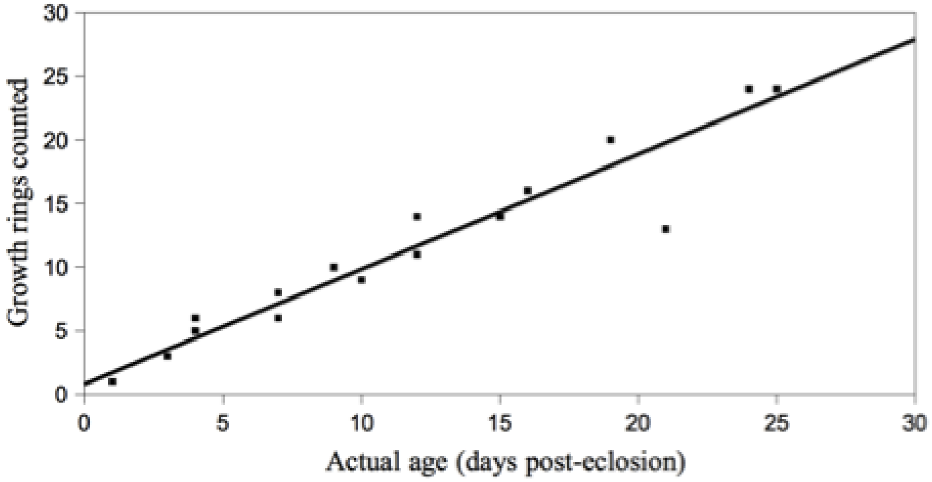

Figure 4.2 Linear regression of growth rings counted on actual age of crickets used in determining the accuracy of the aging method.

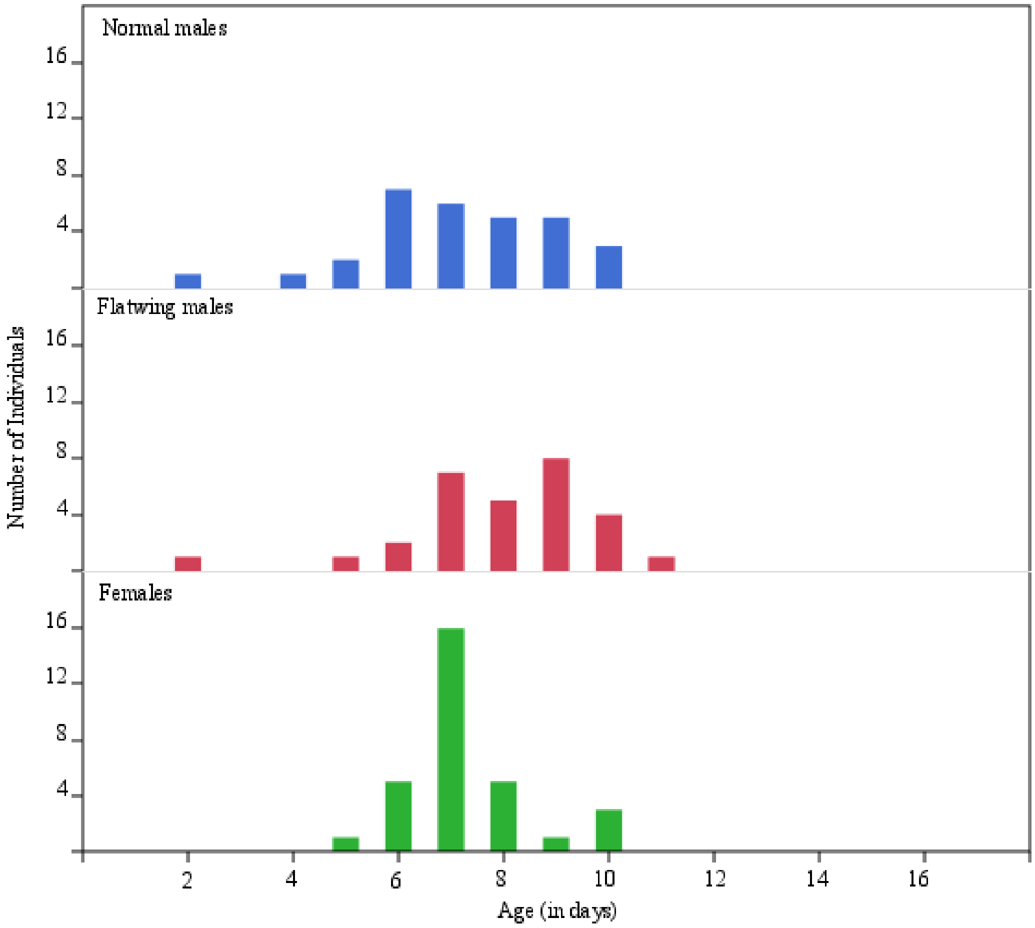

Figure 4.3 Age distributions of normal-winged males, flatwing males, and females in sampling year 2010.

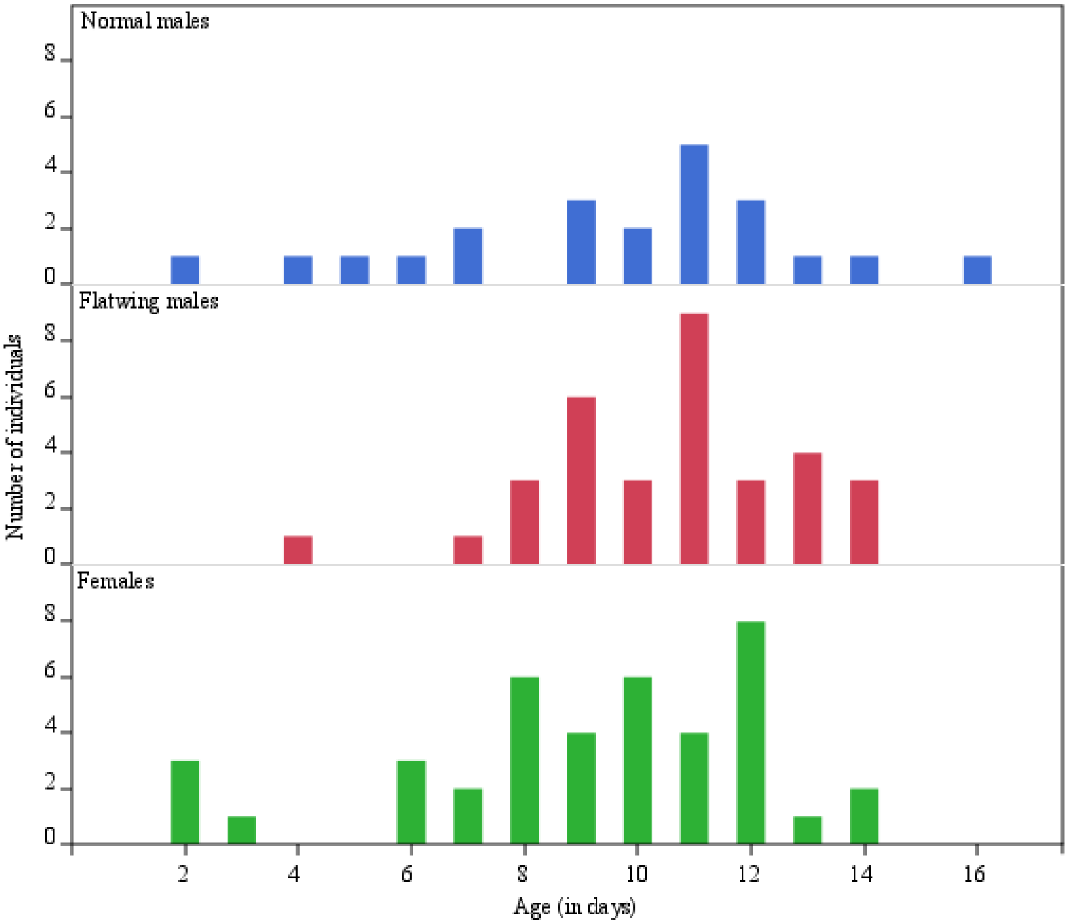

Figure 4.4 Age distributions of normal-winged males, flatwing males, and females in sampling year 2012.

DISSERTATION CONCLUSIONS

While sexual signal loss may be a relatively common occurrence (Wiens 2001), we often do not have the opportunity to observe the consequences of the evolutionary loss of sexual signals because they rarely occur on a contemporary timescale (Svensson and Gosden 2007). In this dissertation, I examined the potential effects of such a loss on populations of the field cricket *Teleogryllus oceanicus*. The loss, while important in and of itself, also alters the acoustic landscape experienced by all crickets, potentially changing perceptions of population density. As such, signal loss in this species may affect the physiology, morphology, and behavior of individuals (Bailey and Zuk 2008, Bailey et al 2010, 2011, Thomas et al 2011). This dissertation provides evidence that the crickets are sensitive to and respond to the change in acoustic environment that results from the spread of flatwing mutation.

I investigated the role acoustic environment plays in modulating perceived sperm competition risk and male reproductive investment (chapter one). Consistent with sperm competition game theory (Parker et al 1997, Simmons 2001), males reared in song-rich environments mimicking a dense population, and thus, a greater risk of sperm competition, produced ejaculates with greater sperm viability, but did not achieve greater fertilization success. That males did not achieve greater fertilization success was not entirely unsurprising, given that females of this species can bias paternity toward specific males (Simmons and Beveridge 2010); however, such a result argues that future studies take into account both male reproductive investment and female choice in order to fully understand the fitness consequences of postcopulatory sexual selection.

I also examined how the altered acoustic landscape affected behavioral displays of aggression. Males reared in silent environments, mimicking a low population density, were more aggressive than males reared in song-dense environments (chapter two), consistent with previous work in two closely related species, *G. bimaculatus* (Iba et al 1995, Nagamoto et al 2005) and *G. integer* (DiRienzo et al 2012). This provides further evidence that crickets, and especially males, are sensitive to changes in the social environment and adjust behavior in response (see also Kasumovic and Brooks 2011).

Because communication is this species is multimodal, I looked for potential signal compensation after the loss of a sexual signal (chapter three). Other species may mitigate the temporary loss of a signaling modality by utilizing other modalities that remain available (Candolin 2003, Hebets and Papaj 2005), such as switching to olfactory signalling in dark conditions, which render visual signals ineffective (Denoël and Doellen 2010). However, little is known about whether organisms may compensate for the permanent loss of a signal modality by switching to another, pre-existing modality. I used a female response assay to determine whether flatwings compensated for a lack of acoustic signalling through chemical cues, but found that females did not preferentially respond to the chemical cues of flatwing males to those of normal-winged males (chapter 3).

Finally, I investigated the potential benefits of the flatwing mutation (chapter four). Normal-winged males are subject to lethal parasitism from the acoustically-orienting fly *O. ochracea* (Zuk et al 1993), and this parasitism reduces male lifespan within a population (Simmons and Zuk 1994). Because *O. ochracea* is unable to locate flatwing males, these males should be free from parasite-induced mortality and experience a longer lifespan than their normal-winged counterparts. By using a method to accurately age adult field-caught crickets (Zuk 1987, Simmons and Zuk 1994), I was able to determine the mean ages of females, normal-winged males, and flatwings at a field site where flatwing and normal-winged males exist in roughly equal numbers. Contrary to my predictions, flatwings were not older than normal-winged males. These results may be confounded by a shift in flatwing behavior; flatwings act as satellites to the few remaining calling males (Zuk et al 2006), displaying phonotaxis toward calling males. This phonotaxis may expose flatwings to increased predation (*sensu* Simmons and Zuk 1994), thereby reducing the benefits of the protection they gain from being unable to produce calls.

In summary, the consequences of evolutionary change may be manifold and varied. In this dissertation, the evolutionary loss of a sexual signal seems to exert a large influence on the social environment experienced by *T. oceanicus*, and this altered social experience affects the behavior and reproductive investment of males in particular (see also Bailey and Zuk 2008, Bailey et al 2010, 2011, Thomas et al 2011). I did not find any evidence of signal compensation, nor any effects of the flatwing mutation on the mean age of males. Future studies will continue to explore potential effects of the flatwing mutation, as well as consequences of the shift in social experience.

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