



# Is it the Song or the Singers? Acoustic and Social Experiences Shape Adult Reproductive Tactics and Condition

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## Abstract

When sexual signals are perceived during growth and development they can provide information regarding the social conditions likely to be encountered as an adult. Perception of cues related to the presence and density of future mates and potential competitors can result in altered adult phenotypes. Previous studies have shown that adult male *Teleogryllus oceanicus* field crickets from a Kauai, Hawaii population reared alone and without hearing conspecific song are more phonotactic than those reared with song. These naïve males also reduce investment in body size and immunity. Here we examined whether another source of population density information, the presence of other males, affects behavior, size, and immunity. Specifically, we examined satellite behavior as evidenced by strength of phonotaxis, body condition, and immune response in males reared singly and in groups in the presence and absence of conspecific song. Body condition did not vary with rearing density, and immune response did not vary with either acoustic environment or rearing density. Interestingly, group-housed males were more phonotactic than singly-housed males. This pattern was largely driven by the low levels of phonotaxis exhibited by males that were singly-housed in the presence of conspecific song. These findings suggest that males respond to social cues in addition to conspecific song, but that these cues do not necessarily provide concordant information.

**Keywords** Behavioral plasticity · density dependent prophylaxis · sexual selection · satellite behavior · signal loss · *Teleogryllus oceanicus*

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## Introduction

Cues received during growth and development can provide valuable information regarding the conditions that individuals are likely to encounter as adults (West-Eberhard 2003; Beldade et al. 2011; Kasumovic and Brooks 2011). Selection should be strongest for the ability to respond adaptively to cues that provide information having high fitness consequences. Social cues from the environment, including exposure to sexual signals and physical contact with conspecifics, can inform immature individuals of the intensity of resource competition. Such information may include the presence and density of future mates and disease transmission risk, with higher population densities more likely to spread infectious disease (Wilson and Cotter 2009; Kasumovic and Brooks 2011; Lemaître et al. 2011). These sexual and social cues detected prior to sexual maturity can trigger plastic developmental mechanisms that result in locally adaptive adult phenotypes for a wide range of traits, including mating behaviors and reproductive investment (Tan et al. 2004; Bailey and Zuk 2008; Bailey et al. 2010), adult body size (Bailey et al. 2010; Niemelä et al. 2012), growth rate (Lihoreau and Rivault 2008; Niemelä et al. 2012), and immunocompetence (Barnes and Siva-Jothy 2000; Bailey et al. 2011; Niemelä et al. 2012).

Multiple cues can provide information about a single environmental factor (e.g., population density), and these cues may be detected across multiple sensory modalities including tactile, olfactory/chemical, acoustic, and visual. In some cases, multiple, redundant cues across several modalities are required to transmit the appropriate social information and stimulate an adaptive response (Bretman et al. 2011), while in other cases, a single cue carries the relevant information. For instance, tactile stimulus of the body, particularly the back legs, of the desert locust (*Schistocerca gregaria*) induces a transition from the solitary to gregarious phase while chemical and visual stimuli do not (Hägele and Simpson 2000; Simpson et al. 2001). In many cases, however, either all modalities have been investigated concurrently (Gage 1995) or only a single modality has been manipulated (Bailey and Zuk 2008; Bailey et al. 2010; Rebar et al. 2016; Rebar and Greenfield 2017). Thus, it is often unclear whether the various signalling modalities are supplying redundant or distinctive information.

Male field crickets (Family: Gryllidae) produce a calling song to attract phonotactic, sexually receptive females. However, males also engage in highly plastic alternative mating tactics (AMTs) known as satellite and wandering behaviors (Waltz 1982). When engaging in satellite behavior, males do not sing, but instead position themselves near another calling male and may intercept females attracted to the calling male (Cade 1979). Wandering behavior is not phonotactic in nature but instead simply refers to increased movement through the environment that may improve a male's chances of encountering a receptive female (French and Cade 1989; Hissmann 1990; Cade and Cade 1992). In previous studies, we found that male *T. oceanicus* field crickets alter their propensity to engage in these AMTs depending on their acoustic rearing environment (Bailey et al. 2010; Balenger and Zuk 2015). Specifically, males reared in the absence of conspecific calling song were more likely to engage in satellite and wandering behaviors than were males reared in the presence of conspecific song (Bailey et al. 2010; Balenger and Zuk

2015). In each of these studies crickets were housed in individual containers (singly) so that they could not receive social cues through any modality other than the acoustic environment. More recently, Zuk et al. (2018) found that both males and females are more phonotactic towards playback of conspecific calling song in the wild when they are from a population where calling song is rare.

In many species, adult size and investment in immunity vary with density. An increase in body condition or immune investment under high density conditions has been suggested to be adaptive in environments perceived to contain many competitors (Gibbs et al. 2004) or pathogens (Wilson and Reeson 1998). When males must compete with one another for access to females or other resources, larger size or body condition should increase an individual's likelihood of gaining reproductive success. Furthermore, increased investment in immunity as density increases, or density dependent prophylaxis (DDP), has been shown to be adaptive in species that experience highly variable density conditions (Wilson and Reeson 1998; Wilson and Cotter 2009). For example, Wilson et al. (2002) found that desert locusts exhibit DDP such that individuals reared in high density conditions are more resistant to the entomopathogenic fungus *Matarhizium anisopliae* than those reared solitarily. In past studies of *T. oceanicus*, manipulation of the acoustic rearing environment affected both adult male body condition and immune measures (Bailey et al. 2010, 2011). Specifically, males housed singly and exposed to song had more mass for their body size and greater lysozyme activity than those reared singly but without song (Bailey et al. 2010).

Here we investigated whether other social cues obtained through the physical presence of other males affected three traits important in reproduction and survival in the descendants of *T. oceanicus* crickets originating from the island of Kauai, Hawaii – phonotaxis, body condition, and immune function. This population is of particular interest with respect to acoustic information content due to the relatively recent and rapid emergence of a novel wing phenotype which renders males obligately silent (Zuk et al. 2006). The acoustic environment on Kauai has therefore undergone a dramatic change in the last 15 years due to the high percentage of males (<90%) expressing this “flatwing” morphology.

Our goal was to evaluate whether the acoustic environment is the exclusive or primary mode capable of triggering this behavioral and physiological plasticity, or if there are additional, redundant cues available in the wild that would trigger such phenotypic plasticity under variable density conditions. Specifically, we examined tendency to engage in phonotactic satellite behavior, adult body condition, and immune function of males reared singly and in groups of five with and without conspecific song during the final two weeks of their juvenile stage and the first six days of their adult stage. If a sensory signal in addition to song conveys information regarding population density, we predicted that individuals in treatments where they were reared in groups and/or with song would exhibit weaker phonotaxis, higher immune function, and larger body size in comparison to individuals reared singly and without song. If, on the other hand, no other sensory signal conveys information regarding population density, we predicted crickets reared in the absence of song, regardless of their social environment, would exhibit increased phonotaxis, decreased immune function, and smaller body size than their counterparts reared in the presence of conspecific song.

## Methods

### Colony Origin and Rearing

A colony of *T. oceanicus* from the Hawaiian island of Kauai was established in 2003. This laboratory population was founded by several hundred eggs laid by approximately 12 wild-caught females. The colony is further supplemented approximately annually with eggs from additional wild-caught females and consists of a minimum of 100 breeding adults at all times. Crickets are reared in 15 L containers in incubators (Caron Insect Growth Control Chambers model 6025) at 26 °C and 75% humidity with a 12:12 h photo-reversed light:dark cycle.

### Treatment Groups

Juvenile male crickets were removed from laboratory colony containers housing approximately 40 individuals when they reached their penultimate instar and sex differences become obvious. Although tympanal membranes are not obvious until adulthood, conspecific playback of calling song only during the penultimate instar induces changes in development rate and adult reproductive behavior (Kasumovic et al. 2011, 2012), and last instar juveniles have the sensitivity to hear songs at close range (Ball et al. 1990). Thus, individuals are capable of hearing song during this late juvenile stage. We haphazardly assigned individuals to one of four treatments, with acoustic environment (song or no song) and social environment (1 male or 5 males) as factors. The population being studied includes individuals with an alternative wing morph called flatwing. Wing morph (normal or flatwing) cannot be identified until adult eclosion, so males were assigned to a treatment group without regard to this factor.

In the social environment treatment, crickets were either placed alone in 118 mL containers containing food (Harlan high fiber rabbit diet), a water vial, and egg carton material for shelter, or as groups of 5 juvenile males in 1.8 L containers similarly containing ad libitum food, moist cotton, and egg carton material (Groups - song boxes:  $n = 15$ ; no song boxes:  $n = 15$ ). Containers were then placed in one of two incubators according to their assignment to a ‘song’ or ‘no song’ treatment group. Treatment incubators lined with sound absorbing foam were set at the same temperature, humidity and photoperiod conditions as described above. In the ‘song’ incubator, an average male calling song, constructed from >20 males recorded in the wild (Zuk et al. 2006), played from three CD players simultaneously, with each player connected to two speakers. Broadcasts were offset so as to give the impression that multiple males with average song parameters were calling; song played during the 12 h scotoperiod only. We used an AZ sound meter (model 8922) to measure the sound pressure level (SPL) and set the SPL to 80–85 dB outside the containers, resulting in an SPL inside of the containers of 70–75 dB, which is the typical intensity of the calling song of a male approximately 0.50 m away (Bailey et al. 2010; Simmons et al. 2001). Conditions were identical in the ‘no song’ treatment incubator except that no song ever played from the speakers.

Crickets were monitored daily for their final molt from nymphal stage to adult form, i.e., eclosion. Only crickets that eclosed 14 or more days after being placed in their treatment were included in the study. Group treatment males not meeting such criteria

remained in their treatment until all other males in their group were tested. Singly housed males were removed from the experiment completely when not meeting the timing criteria. Final sample sizes tested from each of three treatments (acoustic environment, social environment, and wing morph) are provided in Table 1.

Within 24 h of eclosion, we removed the scraper from each male's right wing by cutting it with surgical scissors. This procedure eliminates acoustic signalling by sources other than the speakers. Removal of the scraper has no discernible effect on males' ability or tendency to move their wings (Bailey et al. 2008; S.L. Balenger, personal observation). The wings of males with the flatwing morph were also clipped in the same location to control for the effects of the procedure. Males in the group containers did not necessarily eclose on the same day, so we marked each male by writing on the pronotum using a white gel pen (Souffle XPG #950) to distinguish males within the same box from one another. Males in single cups were similarly marked to control for handling and marker effects. Both treatments (acoustic and social) continued until testing day, allowing individuals to experience treatments during late juvenile and early adult stages.

All behavioral and condition measurements were taken six days post-eclosion. Crickets will therefore have undergone exposure to social and acoustic treatments as both late stage juveniles and young adults until they reached sexual maturity. Crickets were tested for phonotaxis response (see below), weighed to the nearest 0.001 g and the length of their pronotums measured to the nearest 0.01 mm, and a small hemolymph sample was collected. Pronotum length was measured twice with digital calipers and the average of the two measures was used in analyses. Hemolymph was collected by puncturing a cricket's abdomen between the third and fourth abdominal sternites and removing 5  $\mu$ l of hemolymph with a micropipetter. We immediately plunged hemolymph samples into 40  $\mu$ l of ice-cold sterilized phosphate-buffered saline (PBS) to prevent activation of immune system enzymes. We kept samples on ice until they could be stored at  $-80^{\circ}$  C.

### Satellite Behavior Trials

Satellite trials were conducted in a 23–24  $^{\circ}$ C room with a red overhead light between the hours of 1200–1700. Due to the photoreversed conditions in the incubators, this

**Table 1** Shown are sample sizes used in this study for each acoustic treatment (song and no song), social environment (solitary and group housed), and wing morph (normal wing and flatwing)

Treatment group	Satellite behavior	Body condition	Lysozyme-like activity	Phenoloxidase
Song, solitary, normal wing	17	16	14	18
Song, solitary, flatwing	21	18	22	20
No song, solitary, normal wing	16	16	13	14
No song, solitary, flatwing	21	21	20	25
Song, group, normal wing	18	18	17	26
Song, group, flatwing	19	18	19	24
No song, group, normal wing	26	24	25	29
No song, group, flatwing	13	13	12	19
Total	151	144	142	175

time coincided with the active scotoperiod. In this study, we mainly followed the method for assessing satellite behavior in Bailey et al. (2010). Here we provide a brief description of the arena and trials, highlighting any differences between Bailey et al. (2010) and this study.

We performed trials in a circular, plastic arena 2.30 m in diameter with 0.56 m high walls. A Sony SRS-M30 speaker was fixed in the centre at floor level and the walls were lined with sound absorbing foam. A 0.50 m radius circle was drawn on the floor around the speaker, which corresponds to the distance of one standard error less than the mean at which satellite males were found near playbacks in the wild (Zuk et al. 2006; Fig. S1). Crumbled leaf litter was loosely spread across the floor of the arena, while still ensuring the visibility of the line demarcating the circle, to provide cover and disguise scent trails. We rearranged the leaf litter between each trial to disrupt odor cues. Four inverted 118 mL plastic cups were spaced equidistant from one another along the edge of the arena and 1.15 m from the centrally located speaker.

To minimize bias, the observer was blind to the treatment group of the individual being tested during behavioral trials. For each trial, a male was placed on top of a fresh square of paper under one of the four cups, where he was allowed to acclimate in silence for 2 min. Starting location was haphazardly chosen, but the same location was never used twice in a row. At the end of the acclimation period, the artificially constructed average male song described above was broadcast from the speaker at 70 dB SPL measured from 0.50 m away. Simultaneous to beginning the playback, we removed the cup covering the male and the 5 min trial began.

We used the SpectatorGo! app for iPads (Biobserve GMBH, St. Augustin, Germany) to record when the male entered the circle 0.50 m from the speaker and how long he spent inside the circle if he did. Individuals that did not enter the circle were common (43% of trials). Results do not qualitatively change as a result of the removal of these data points, therefore we chose to retain these trials in analyses and assigned a value of 300 s to the former measurement (latency to enter the circle) and 0 s to the latter (time spent in circle).

### Lysozyme-Like Activity Assay

We measured lysozyme-like activity, a component of the insect humoral immune response, by testing the ability of sample animals' hemolymph to lyse a solution of the bacteria *Micrococcus luteus* (ACROS Organics) (Adamo 2004). For each sample, we added 10  $\mu$ l of hemolymph and PBS solution to a well in a 96-well plate. We then added 90  $\mu$ l of a 0.35 mg/ml *M. luteus* solution. We measured absorbance at 490 nm every 10 min for 2 h using a plate reader (BioTek, Synergy HT). Greater decreases in absorbance indicate higher activity as lytic enzymes break down the bacteria in the solution (Fedorka et al. 2004; Bailey et al. 2011).

### Phenoloxidase Assay

Phenoloxidase (PO) is a key enzyme in the insect melanization response, which surrounds pathogens or parasites in melanin to immobilize them (Schmid-Hempel 2005). To determine how much PO was potentially available in the hemolymph of our crickets, we quantified active PO as well as activating and quantifying its inactive

form: pro-PO. Pro-PO is cleaved to form PO after immune challenge. Greater activation of pro-PO correlates with higher immune system activity (Adamo 2004).

For each sample, we combined 5  $\mu\text{l}$  of hemolymph and PBS with 7  $\mu\text{l}$  of 1.3 mg/ml  $\alpha$ -chymotrypsin (Sigma Aldrich) in a 96 well plate (Sigma Aldrich). In each plate, the last row of wells contained no hemolymph and served as a set of controls. This solution was pipetted to mix and incubated at room temperature for 20 min. Next, we added 90  $\mu\text{l}$  of a 15 mM solution of L-dihydroxyphenylalanine (L-DOPA, ACROS Organics) in 1X PBS to each well. We measured melanin formation (indicated by increased absorbance) using a plate reader (BioTek, Synergy HT) for 120 min at 490 nm. Readings were taken every 10 min over 2 h. Greater increases in absorbance indicate higher PO activity (Fedorka et al. 2004; Adamo 2004; Shoemaker et al. 2006; Bailey et al. 2011).

## Analysis

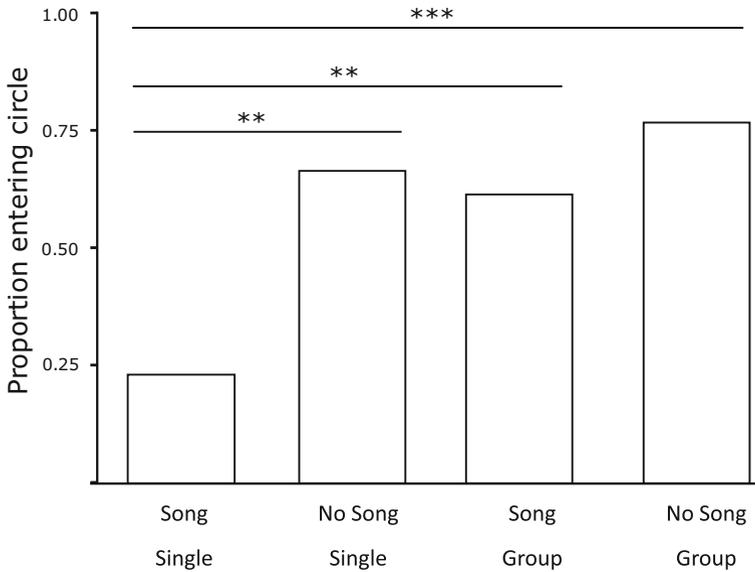
Fully factorial backwards regression tests were used to evaluate whether acoustic environment (song or no song), social environment (singly housed or group housed with 4 other males), or wing morph (normal wing or flatwing) explained 1) how long it took for a male to enter the 0.50 m radius circle, 2) how much time a male spent inside that circle, 3) body condition, and 4) immune condition (lysozyme and phenoloxidase assays) with minimum AICc as the selection criterion. As there is some disagreement in the literature regarding the use of mass-size residuals versus mass alone (Green 2001; Schulte-Hostedde et al. 2005), we analysed both. The results were not qualitatively different, so we only present the analysis of residual body condition. We also used a fully factorial logistic regression with effect likelihood ratio tests to evaluate whether these same factors of interest (acoustic environment, social environment, and wing morph) predicted the likelihood that males would enter the 0.50 m radius circle or not. Body condition was estimated using the residuals from a regression of mass on pronotum length.

To show complete models with  $p$ -values, fully factorial least squares effect (LSE) tests were subsequently used to evaluate the effect of acoustic environment, social environment and wing morph on these same response variables. Box ID was included in all models as a random effect using a REML estimation. All statistical analyses were performed in JMP Pro v. 11. Data is deposited in DataDryad under the identifier <https://doi.org/10.5061/dryad.t2.14v1>.

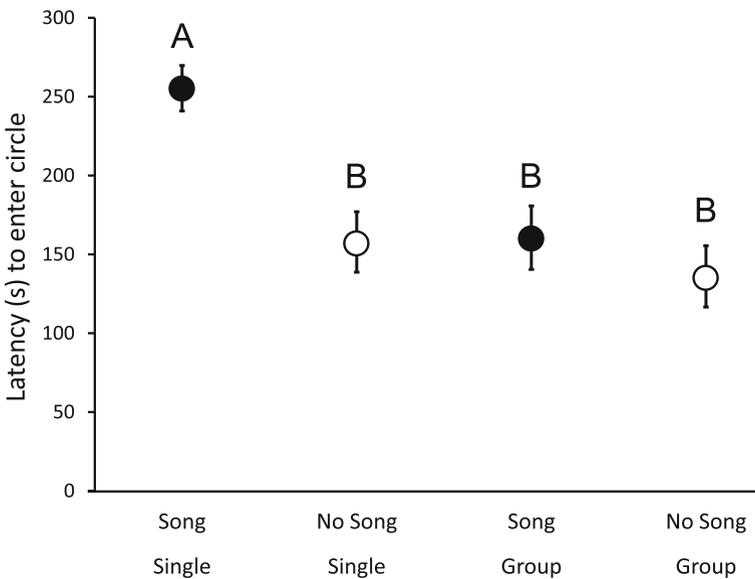
## Results

### Satellite Behavior

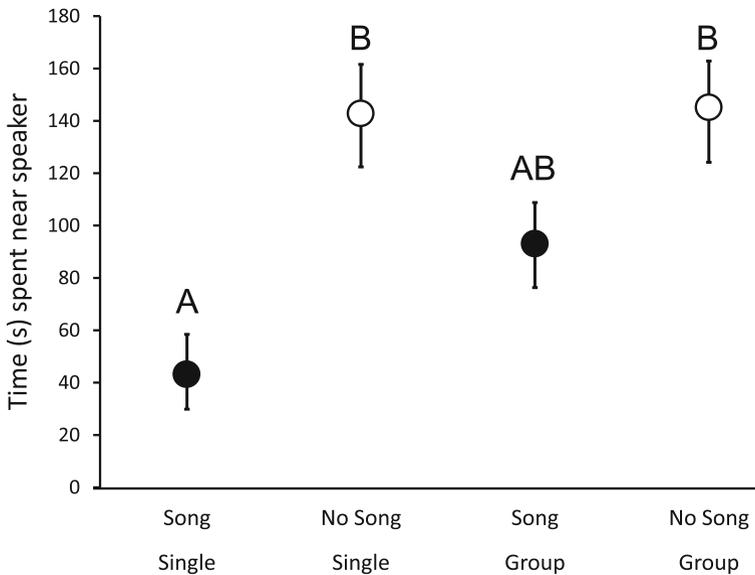
Acoustic rearing environment was a significant predictor for all measures associated with satellite behaviors. Relative to crickets reared in the presence of conspecific calling song, males reared without song were more likely to move within 0.50 m of the playback speaker during a 5-min trial, moved more quickly to within 0.50 m of the playback speaker, and spent more time within 0.50 m of the playback speaker over the course of the trial (Figs. 1, 2, and 3; Tables 2, 3 and 4). Actual social rearing environment (singly versus group housed) was also a significant factor in predicting



**Fig. 1** The effect of acoustic rearing environment (with calling song, “Song”; without calling song “No Song”) and the physical presence of other crickets (without other crickets, “Single”; raised in a group of 5 crickets, “Group”) on the probability that a male came within 50 cm of a synthetic conspecific playback recording. Results of post hoc least squares means pairwise comparisons are indicated (\*\*  $p < 0.001$ ; \*\*\*  $p < 0.0001$ )



**Fig. 2** The effect of acoustic rearing environment (with calling song, “Song”; without calling song “No Song”) and the physical presence of other crickets (without other crickets, “Single”; raised in a group of 5 crickets, “Group”) on adult male latency to come within 50 cm of a synthetic conspecific playback recording. Shorter latencies are associated with a satellite male phenotype. Results of post hoc Tukey HSD tests are shown above means  $\pm$  S.E.



**Fig. 3** The effect of acoustic rearing environment (with calling song, “Song”; without calling song “No Song”) and the physical presence of other crickets (without other crickets, “Single”; raised in a group of 5 crickets, “Group”) on the amount of time adult males spent within 50 cm of a synthetic conspecific playback recording. More time spent near the speaker is associated with a satellite phenotype. Results of post hoc Tukey HSD tests are shown above means  $\pm$  S.E.

whether and how quickly males would engage in satellite behaviors, but it was not significantly related to how long they would do so. Males reared in groups were more likely to move within 0.50 m of the playback speaker and moved more quickly to within 0.50 m of the playback speaker, but they spent a similar amount of time within 0.50 m of the playback over the course of the 5 min trial (Figs. 1, 2, and 3; Tables 2, 3

**Table 2** Results of a fully factorial logistic regression with effect likelihood ratio tests examining the effects of developmental acoustic environment, social environment, and wing morph on likelihood of moving within 0.50 m of a synthetic conspecific playback recording

Model $r^2 = 0.13$	df	Likelihood Ratio $X^2$	<i>p</i>
Acoustic environment	1	10.77	<b>0.001</b>
Social environment	1	7.03	<b>0.008</b>
Wing morph	1	0.10	0.75
Acoustic x social	1	3.89	<b>0.05</b>
Acoustic x morph	1	0.21	0.65
Social x morph	1	0.80	0.37
Acoustic x social x morph	1	0.98	0.32
Full model	7	26.66	<b>0.0004</b>

The area within 0.50 m of the recorded song represents the region within which males of this species engage in a satellite mating tactic

*p*-values  $\leq 0.05$  are bolded

**Table 3** Results of fully factorial least squares effects tests examining the effects of developmental acoustic environment, social environment, and wing morph on latency to move within 0.50 m of a synthetic conspecific playback recording

Model $r^2 = 0.39$	df	<i>F</i>	<i>p</i>
Acoustic environment	1	5.97	<b>0.02</b>
Social environment	1	5.74	<b>0.02</b>
Wing morph	1	0.94	0.34
Acoustic x social	1	5.15	<b>0.03</b>
Acoustic x morph	1	0.10	0.75
Social x morph	1	1.54	0.22
Acoustic x social x morph	1	0.74	0.39

The area within 0.50 m of the recorded song represents the region within which males of this species engage in a satellite mating tactic

*p*-values  $\leq 0.05$  are bolded

and 4). Wing morph (normal versus flatwing) did not significantly predict any behavioral response, either on its own or when entered into models as part of an interaction term (Figs. 1, 2, and 3; Tables 2, 3 and 4).

The interaction between acoustic and social environment was a significant predictor of both the likelihood that a male moved within 0.50 m of the playback during the 5 min trial and a male's latency to move within 0.50 m of the playback (Figs. 1 and 2, Tables 2 and 3). *Post hoc* examination of the differences of Least Squares Mean for all possible pairwise comparisons show that this is primarily due to males reared both singly and in the presence of song being less likely to move within 0.50 m of the playback speaker than any of the other groups (Fig. 1). No other interactions between acoustic environment, social environment, or wing morph significantly affected either of these behaviors nor the amount of time males spent within 0.50 m of the playback speaker (Figs. 1, 2, and 3; Tables 2, 3 and 4).

**Table 4** Results of fully factorial least squares effects tests examining the effects of developmental acoustic environment, social environment, and wing morph on the amount of time spent within 0.50 m of a synthetic conspecific playback recording

Model $r^2 = 0.23$	df	<i>F</i>	<i>p</i>
Acoustic environment	1	11.99	<b>0.001</b>
Social environment	1	0.80	0.37
Wing morph	1	0.23	0.63
Acoustic x social	1	3.15	0.08
Acoustic x morph	1	0.51	0.47
Social x morph	1	0.83	0.36
Acoustic x social x morph	1	2.47	0.11

The area within 0.50 m of the recorded song represents the region within which males of this species engage in a satellite mating tactic

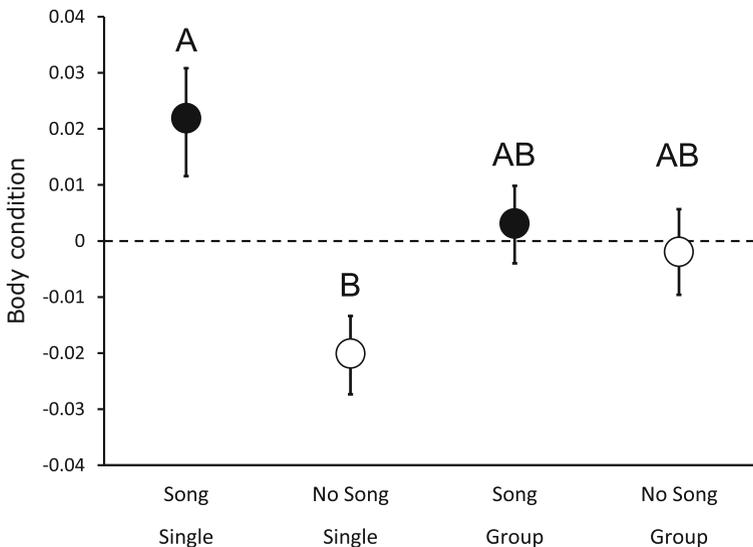
*p*-values  $\leq 0.05$  are bolded

## Body Condition and Immune Response

Males reared in the presence of conspecific calling song had significantly greater body condition than those reared without song; this appears to largely be driven by the difference in body condition seen between singly reared males from the two acoustic treatments (Fig. 4; Table 5). Immune response as measured by phenoloxidase and lytic activity did not significantly differ with respect to acoustic environment, social environment, wing morph, or any interaction terms (all  $p > 0.05$ ; Tables S1 & S2).

## Discussion

Laboratory-bred, singly housed *T. oceanicus* field crickets from Kauai, Hawaii exhibit increased phonotaxis and decreased body size when raised in the absence of conspecific song (Bailey and Zuk 2008; Bailey et al. 2010, this study). In the wild, responsiveness to playback by *T. oceanicus* on the Hawaiian Island of Kauai similarly indicates increased phonotaxis by crickets living in this low density, nearly caller-free population (Zuk et al. 2018). Here we manipulated aspects of the social environment of male *T. oceanicus* derived from the Kauai population with the goal of testing whether adult phonotactic behavior and physiological condition respond to potential density cues perceived via non-auditory sensory modalities. We first validated previous findings that singly-housed males reared in the absence of conspecific song were more phonotactic and had smaller body size relative to singly-housed males reared in the presence of conspecific song (Bailey et al. 2010). Contrary to previous findings, though, we found no significant difference in immunity of singly-housed males with respect to acoustic rearing



**Fig. 4** The effect of acoustic rearing environment (with calling song, “Song”; without calling song “No Song”) and the physical presence of other crickets (without other crickets, “Single”; raised in a group of 5 crickets, “Group”) on adult male body condition. Condition is estimated using the residuals from a regression of body mass on pronotum length. Results of post hoc Tukey HSD tests are shown above means  $\pm$  S.E.

**Table 5** Results of fully factorial least squares effects tests examining the effects of developmental acoustic environment, social environment, and wing morph on body condition as estimated by the residuals from a regression of mass on pronotum length

Model $r^2 = 0.51$	df	<i>F</i>	<i>p</i>
Acoustic environment	1	5.77	<b>0.02</b>
Social environment	1	0.45	0.51
Wing morph	1	1.93	0.17
Acoustic x social	1	0.59	0.45
Acoustic x morph	1	0.05	0.82
Social x morph	1	0.96	0.33
Acoustic x social x morph	1	0.20	0.66

*p*-values  $\leq 0.05$  are bolded

environment (Bailey et al. 2011). To examine whether non-auditory sensory modalities also influence plasticity in these traits, we also housed males in groups of five (group-housed) and reared them either in the presence or absence of conspecific song. Among group-housed males, there was no significant difference in phonotactic behavior, body condition, or immune measures regardless of their acoustic environment. This finding suggests that high density social conditions provide additional cues that are preferentially used over acoustic cues in determining the adult phenotype. *Post hoc* tests revealed that singly-housed males reared in the presence of song were significantly less phonotactic, and thus less likely to behave as satellite males, than were the other three treatment groups; no such difference was found for body size or immunity.

In the wild, field cricket population density can vary dramatically over time, at least in some species (Hissmann 1990), and reliable cues providing information with respect to numbers of mates and competitors have the potential to be adaptive under such variable conditions. Multiple studies investigating response by juvenile and adult field crickets to exposure of average adult song vs. no song at all have identified phenotypically plastic responses to this typically reliable cue for population density and the availability of potential mates (Bailey and Zuk 2008; Bailey et al. 2010, 2011; Balenger and Zuk 2015; Kasumovic et al. 2011, 2012). In most studies investigating field cricket phenotypic plasticity in response to acoustic cues, individuals are isolated prior to sexual maturity. This is done in part to facilitate determining the age of individuals following eclosion and to eliminate potentially confounding factors that may arise through social interactions. Thus, other cues of population density that could potentially be available in the wild (e.g., tactile and chemical cues) are not available to the animals under study. In the current study we manipulated aspects of the social environment of male *T. oceanicus* field crickets with the goal of testing whether adult reproductive behavior and/or condition respond to information regarding density that could be perceived via other sensory modalities. While the acoustic environment had a significant effect both on body size and phonotactic behaviors, this effect was largely driven by relatively lower phonotaxis by males reared singly and in the presence of conspecific calling song. One possibility is that adaptive plasticity in response to acoustic cues may be under selection in this population within the context of the current relatively low population density conditions being experienced on Kauai since a novel wing

morph rendering males silent became common in the population approximately 15 years ago (Zuk et al. 2006, 2018). One way to address this would be to compare responses by Kauai crickets to those of populations with higher natural densities that do and do not have obligately silent males. At this time there are no known silent populations that regularly experience high densities of conspecifics. An alternative possibility is that there is an effect of acoustic rearing environment on satellite behavior of group-housed males, but this effect is not seen until males are older than those tested here. Bailey et al. (2010) found that the strength of phonotaxis increased among males reared singly and without song between the ages of 6 d.o. (used in this study) and 13 d.o.

Zuk et al. (2006) first reported the widespread occurrence of a novel wing polymorphism among male *T. oceanicus* on the island of Kauai, Hawaii. Males with this wing phenotype, known as “flatwing,” lack the forewing stridulatory structures necessary to produce normal song. This phenotype has likely become established due to strong selective pressure exerted on calling behavior by *Ormia ochracea*, an acoustically-orienting parasitoid fly that is also present on the island. The frequency of this sex-linked, sex-limited trait (Tinghitella 2008) has remained relatively stable in the years since the discovery, with >90% of males in the population exhibiting the flatwing phenotype. Thus, for over a decade, *T. oceanicus* on Kauai have experienced an unusual acoustic environment in which very few individuals can produce song. In such an environment, an increased propensity to engage in non-singing alternative mating tactics, like satellite and wandering behaviors, could be adaptive. Although we might expect that the relative fitness benefits of engaging in such behaviors would be greater for flatwing males, wing morph has not been found to be related to mating behaviors of males from Kauai (Bailey et al. 2010, Balenger and Zuk 2015, this study). Instead, plasticity in adult mating tactics *per se* in response to acoustic cues received during juvenile development and sexual maturation likely have allowed for the persistence of this population in the wild following the dramatic loss of its sexual signal (Zuk et al. 2014).

Although satellite behaviors exhibited by singly housed males in this study do confirm previous results (Bailey et al. 2010), our finding that group-housed males, regardless of acoustic rearing environment, exhibit phonotaxis similar in strength to that of singly-housed males reared without song suggests that, when density is high enough for social interactions to be common, information from alternative cues may be prioritized over the presence or amount of song.

When density is high, regardless of acoustic environment, males may be more likely to encounter one another. This could lead to increased satellite behavior, as well as an increase in other types of male-male interactions. Zuk et al.’s (2018) finding that Kauai individuals in the wild exhibit greater phonotaxis to conspecific calling song relative to other Hawaiian populations provides support that laboratory studies of singly-housed Kauai males do indeed reflect behaviors for this population in the wild.

Bailey et al. (2010) found that males housed singly and exposed to song had more mass for their body size than did those reared similarly but without song, and this effect was stronger as males aged. Studies of *T. commodus* have also found an effect of acoustic rearing environment on mass of individually housed adult crickets (Kasumovic et al. 2011; DiRienzo et al. 2012). Specifically, Kasumovic et al. (2011) found that singly-housed *T. commodus* males developing in the presence of the

synthetic songs of three males matured faster, reached smaller adult size, and engaged in more calling behavior than did those developing in the presence of the synthetic songs of 12 males. Bailey et al. (2010) suggested that increased body size was likely an adaptive response to an environment perceived to contain many competitors, but it is unclear what mechanism (e.g., activity levels, food intake, timing of eclosion) is driving this difference in body size in response to acoustic rearing environment. Reduced food intake, for example, could have downstream effects on other aspects of quality affecting fitness and this possibility should be investigated in the future. On the other hand, we did not find a difference in condition for males reared in groups. Larger body size would seem to be adaptive for males living in such high density conditions if aggressive encounters were increased. One possibility is that males in high density environments are more tolerant of other males and so aggressive interactions are reduced, thus making investment in relatively large size unnecessary. Simmons (1986) found that *G. bimaculatus* male aggression decreased with increased population densities. In that same species, Iba et al. (1995) found that males reared in crowded conditions were not only smaller than those reared in solitary, but they expressed higher levels of octopamine, a hormone associated with increased aggression. Given that male crickets are more aggressive when reared alone than when reared in a group, it would be interesting to examine how population density and acoustic environment interact in their effects on male-male aggression. A second possibility is that there is a population density threshold above which males do not increase their mass. We did not monitor interactions between males within boxes, so it is also possible that males in the group treatments could not gain as much mass due to greater physical activity or altered physiological responses due to the close proximity of other males.

In a previous study, Bailey et al. (2011) found that acoustic rearing conditions of singly-housed *T. oceanicus* males significantly predicted lysozyme-like activity but not PO activity. Specifically, males reared in the absence of song showed reduced lytic activity in comparison to those reared with song (Bailey et al. 2011). If males use song as a cue to population density and lower density populations have lower parasite transmission, then males that invest less in their immune system under these conditions would have additional resources that could be invested in reproduction. We hypothesized that male immune investment would respond to cues other than song, and that interactions with other crickets, regardless of the presence of song, would lead to a robust lysozyme response. Surprisingly, we found no significant difference between any of the treatments (acoustic environment, social environment, or their interaction) for either lysozyme-like activity or PO activity. Thus, not only did we not find the predicted response, we also did not replicate the finding in Bailey et al. (2011). One possibility for our inability to find an effect of song on immune response in singly housed males could be that the sample size in this study was relatively small. Bailey et al. (2011) examined lytic activity in a total of 395 males (188 reared in the absence of song and 207 reared in the presence of song), while we obtained lytic activity measures for 142 males (70 reared in the absence of song and 72 reared in the presence of song). However, given the effect sizes of the current study, a posthoc power analysis revealed that we would need approximately 3000 samples to reach 80% power. Therefore, it is unlikely that there was an effect of acoustic environment on lytic activity among the crickets in the current study.

Due to the stochastic nature of population density and sex ratio in the wild, populations are likely to experience a wide variety of social environments over time. In several species of field cricket studied with respect to alternative mating tactics and population density, researchers found that males have greater mating success by reducing the time spent calling when density is high (*Gryllus integer*, Cade and Cade 1992) and that calling is more common than non-calling behaviors by males in low-density contexts (*G. campestris*, Hissmann 1990). In these studies, satellite and wandering behaviors are interpreted as being adaptive in high density environments because calling is energetically costly and less effective when many competitors are also calling. Interestingly, *G. integer* is subject to parasitism by *O. ochracea* while *G. campestris* is not, suggesting that such density-dependent responses are not limited to populations under selection by this acoustically orienting parasitoid. In light of these studies, it would be of interest to examine a greater variety of density conditions for *T. oceanicus* from Kauai. For example, an increase in container size for group-housed males may more accurately reflect conditions encountered in the wild while still providing a relatively high density social environment. It is also possible that, similar to wandering behaviors, acoustically-driven plasticity in male satellite behavior is specific to Kauai crickets evolving in an environment that consistently contains very few calling males (Balenger and Zuk 2015).

It is common practice to house insects and other animals in either individual containers to facilitate ease of identifying individuals and controlling the social environment or in unusually high-density conditions for routine laboratory population maintenance. Such conditions may have heretofore unforeseen consequences on plastic adult characteristics. Our results suggest that even when studying animals that are not highly social we should be cautious when altering social conditions.

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