

Female crickets reared in silence exhibit risk-sensitive behavior in response to the
preferred song

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Dedication

This dissertation is dedicated to my father, Shahin Agalarov.

Abstract

Female choice is influenced by many environmental cues that get experienced during different stages of female development. Cues experienced in juvenile stage may have larger impact on female mate choice than mating decisions in response to cues experienced during adult mate choice. To examine how juvenile acoustic rearing interacts with immediate perception of risk in shaping female mate preference, we used the system of Pacific field cricket (*Teleogryllus oceanicus*). Females raised in silence were predicted to be more responsive under conditions associated with low risk without discriminating across song models. Females raised in the presence of song were expected to exhibit preference for a song model under conditions associated with low risk. Our results showed no interaction for females raised in the presence of song; females raised in silence exhibited preference for a song model under conditions associated with risk.

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Female crickets reared in silence exhibit risk-sensitive behavior in response to the preferred song

Choosing and attracting mates is of central importance in the lives of many sexually reproducing species (Bonachea & Ryan, 2011). However, mating signals are often exhibited in environments that may expose mate-seeking individuals to predation (Cade, 1975; Ryan et al., 1982; Zuk & Kolluru, 1998). Predation risk can impose a strong cost to males with conspicuous traits that attract females (Cade, 1975; Bailey & Haythornthwaite, 1998; Lloyd & Wing, 1983; Walker, 1964). At the same time, females responding to those conspicuous signals also can expose themselves to predation (Baugh & Ryan, 2010; Bonachea & Ryan, 2011; Hughes et al., 2012; Jennions & Petrie, 1997; Sakaluk & Belwood, 1984; Magnhagen, 1991; Wing, 1988).

Such perception of increased risk has been associated with the decreased expression of mating preferences (Hedrick & Dill, 1993; Pilakouta & Alonzo, 2013). Under perception of risky conditions, females might even reverse the direction of their preference, since mate searching can increase the chance of encountering predators (Pilakouta & Alonzo, 2013). For example, in the field cricket, *Gryllus integer*, females generally prefer males that call in long bouts (Hedrick & Dill, 1993). However, females decrease their preference for long bout if faced with the decision to move across open area towards the speaker and instead settle for short calling bout under cover which is associated with presumably lower perceived risk of predation (Hedrick & Dill, 1993).

Along with the empirical findings, theoretical models also predict ‘cost-reducing tactics’ in female reproductive strategies in association with females’ perception of risk (Kirkpatrick, 1996; Lima & Dill, 1990). Females are expected to adjust their preferences

under risky conditions to increase their likelihood of survival (Whitman & Agrawal, 2009). Rather than treating mate preference as an invariant trait, in these models females exhibit behavioral plasticity by adjusting their mating preferences in response to risk related environmental factors (Fowler-Finn & Rodriguez, 2012; Whitman & Agrawal, 2009).

Researchers identify two levels of behavioral plasticity that produce variation in phenotypes optimally matching environmental conditions (Fowler-Finn & Rodriguez, 2012; Snell-Rood, 2012; Snell-Rood, 2013; West-Eberhard, 1989). Snell-Rood (2013) defines these two levels of plasticity as developmental and activational types based on the levels of biological organization involved in the coordinated response to the environment. According to this framework, the mechanism responsible for developmental plasticity spans across multiple levels of biological organization (genotypic, biochemical, physiological, morphological) and represents a slower integrated refinement of traits that affect survival, such as the formation of limbs or muscles. The mechanism responsible for activational plasticity involves the expression of different behaviors in different environments and ensures immediate response to environmental factors, such as hiding in response to risky conditions (Snell-Rood, 2013). Despite the importance of understanding the consequences of plasticity in response to varying environmental conditions, a limited number of studies explored the combined effect of developmental plasticity and activational plasticity (Snell-Rood, 2013). Here, we explored the interaction between rearing-induced developmental plasticity and activational plasticity induced by risky conditions.

We have a unique opportunity to explore the intersection of developmental and activational plasticity induced by risk in the Pacific field cricket, *Teleogryllus oceanicus*, a species native to Australia and the Pacific that has been introduced to the Hawaiian Islands (Zuk et al., 1993). Like other crickets, male *T. oceanicus* use calls to attract females (Zuk et al. 1993). This species produces a two-part calling song, with a trill-like “long chirp” of 3-9 pulses (wing closures) followed by a series of paired pulses, the “short chirps” (Zuk et al., 2001). Previous work on *T. oceanicus* in Hawaii showed that females prefer songs with a higher proportion of long chirp (Bailey & Zuk, 2008).

In the Hawaiian Islands (Oahu, the Big Island of Hawaii and Kauai), *T. oceanicus* is parasitized by the acoustically-orienting fly, *Ormia ochracea* (Zuk et al. 1993, Zuk et al. 2018). *O. ochracea* locates a male cricket using the calling song and deposits larvae inside the cricket, killing it upon emergence (Zuk et al., 1993). Parasitization imposed selection against male callers on the three Hawaiian Islands, which led to the evolution of a new ‘flatwing’ male morph (Zuk et al. 2006). ‘Flatwing’ morphs lack the critical wing structures necessary to produce calling song. Although present in different proportions on all three Hawaiian islands, this flatwing morphology is seen in over 90 percent of male crickets on Kauai resulting in a nearly silent environment on this island (Zuk et al. 2018; Zuk et al., 2006). This newly evolved wing morph protects males from the fly, but poses an obvious mate attraction problem (Zuk et al., 2006; Zuk et al., 2018). How have the flatwings persisted? Previous work on this system suggests that both developmental plasticity induced by rearing helped the silent males become established. Several experiments that manipulated the acoustic environment in which the crickets were reared showed that growing up in silence, as would be expected

for crickets on Kauai since the establishment of the flatwings, influences both male and female behavior. More specifically, males reared in silence are more likely to approach a speaker playing conspecific song (Bailey et al., 2010), and females are less likely to discriminate among playbacks of differentially attractive songs (Bailey & Zuk, 2008). In contrast, females reared hearing conspecific song demonstrate preference for 60 percent long chirp song model and are more likely to discriminate against less preferred song models (Bailey & Zuk, 2008).

These previous experiments examined plasticity that resulted from rearing in environments with and without conspecific song received earlier in development. Since environmental cues received in early stage of development interact with cues received in adult stages, it becomes important to study the influence of such interaction on the manifestation of traits. Because environmental cues received earlier may result in higher integration of mate choice relative to immediate cues such as perception of risk received in adult stage, we manipulated acoustic rearing in the juvenile stage and risk perception in the adult stage to examine how developmental plasticity in mate choice interacts with risk perception during adult choice. We examined the preference of acoustically differentially reared female *T. oceanicus* to playback, under both cover and full exposure, which presumably correspond to respectively low and high levels of risk conditions. Our measure of preference comes from contrasting responses to a preferred and nonpreferred song to different individuals. We hypothesized that the risk associated with exercising mating preferences will cause females to reduce preference for a generally preferred song model in the open area (Hedrick & Dill, 1993; Pilakouta & Alonzo, 2013). At the same time, given the prior evidence of the mediating effect of silent acoustic rearing on female

mate preference, we hypothesized that crickets housed in a silent environment will be generally more responsive to song than crickets reared hearing song (Bailey & Zuk, 2008). If developmental plasticity as a result of acoustic rearing has provided a selective advantage, female crickets reared in silence, which closely resembles current environment on the island of Kauai were expected to be more responsive. They were also predicted to be more likely to respond to playback under cover than across an open area regardless of the song model (Fig. 1).

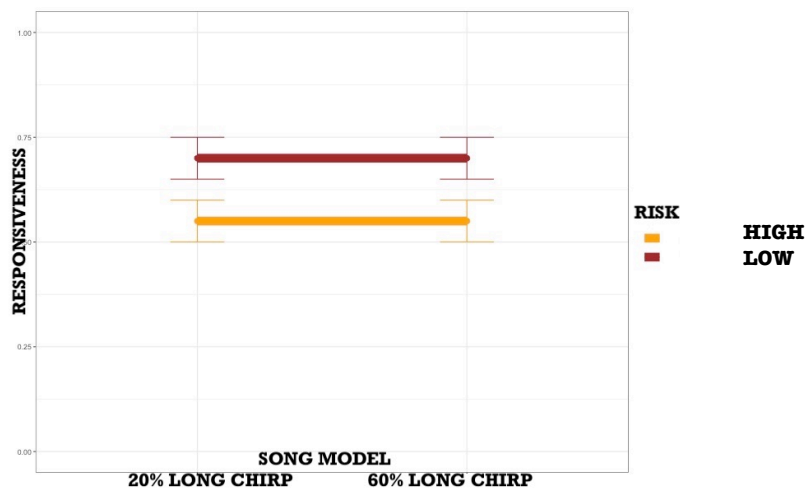


Figure 1. Graphical representation of prediction for No Song reared females

Instead, assessment of song model was predicted to be more important for females hearing conspecific song during rearing. These females were predicted to be more likely to discriminate against a non-preferred song model and base their response to playback song largely as an assessment of song model mediated by risk conditions (Fig. 2). More specifically, we predicted that female crickets in Song environment will be more likely to respond to preferred song model (60 percent long chirp) under conditions associated with low risk.

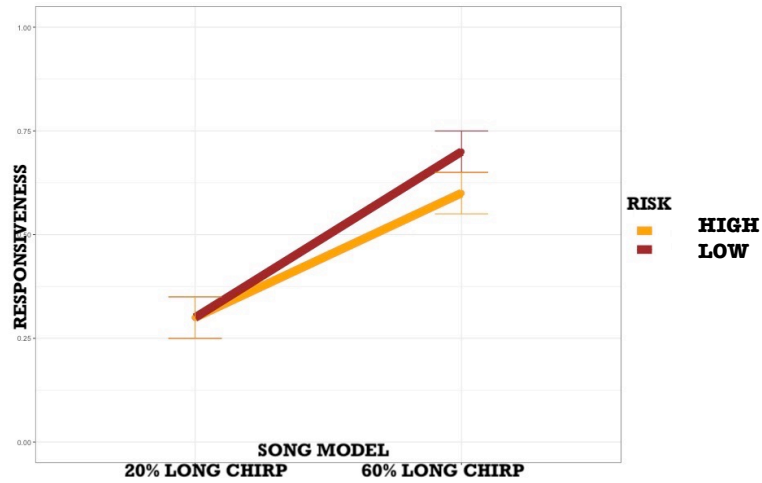


Figure 2. Graphical representation of prediction for Song reared females

Methods

Study system

Experimental manipulation of acoustic rearing.

A *T. oceanicus* laboratory colony was established using eggs from approximately 25 females collected on Kauai in 2006 and supplemented yearly using offspring of wild-caught females so that at least 100 breeding individuals were in the colony at all times. All crickets were reared in 15 l containers that included Fluker’s cricket chow or rabbit chow and water ad-libitum, with egg carton for shelter. Containers were kept in humidity-and temperature-controlled incubators (give brand and model) set at 26C and 75% humidity on a 12:12 photo reversed light: dark cycle. Upon reaching the fourth instar, when sex differences become apparent, females were separated individually into 118 ml plastic containers and randomly assigned to one of two acoustic rearing conditions in the incubators until trials began. The fourth instar is a critical nymphal stage out of five molts in cricket’s life-history that plays a critical role in determining

acoustic plasticity (Huber, Moore & Loher, 1989). Females remained in differential acoustic conditions until the day of the trial. In the No Song environment, crickets were housed without any cricket song. In the Song environment, the female crickets were exposed to generalized male calling song, which was constructed from an average of 20 wild male songs recorded from the population of Kauai island (Zuk et al., 2006) and broadcast inside the incubator from Sony SRS-M30 speakers during the dark phase of the light: dark cycle. Song intensities inside the incubator measured at 70 to 75 dB, which corresponds to a male cricket song at 50 cm (Simmons et al., 2001). To minimize differences in the song exposure levels cups were rotated daily in the Song incubator. Each female was assigned one experimental treatment (as described below) and only tested once. The crickets were tested between June 2017 and January 2018.

Measuring females' response to playback under low and high risk conditions.

Females were tested during their dark cycle, 6-9 days post-eclosion. To mitigate the effect of recent exposure to song, 'prior male' effect, females reared in the Song incubator were removed and placed into the silent incubator 4 hours prior to the experiment on the day of the trial (Wagner et al., 2001). Trials were conducted under red light in a rectangular carton chamber, 116 cm x 30 cm x 35 cm in the anechoic room (Fig. 3). We measured female preference by broadcasting randomly assigned song model, either 60% (preferred) or 20% (non-preferred) long chirp from one of the speakers placed at the end of the longer dimension of the chamber, behind a mesh window. These song models were constructed following the techniques of Simmons et al. (2001). Using software package, Raven v. 1.2, individual long and short chirp pulses were excised from a song recorded in the wild of Kauai island at 25 C, excised pulses were concatenated to

construct two song models that contained 20% and 60% long chirp respectively (Bailey, 2008).

The song was broadcast at the intensity of 65dB at 110 cm. The speakers were alternated to strike balance between speaker location and treatments. To test how perception of risk influences females' likelihood to respond to playback, or mate preference, we randomly assigned presence or absence of cover during each trial. Black wire window mesh was folded to increase the overall complexity and used as cover to simulate low risk conditions. The edges of the folded mesh were attached to thin wooden planks extending almost fully along longer dimension of the chamber (113 cm) and supporting the wire mesh suspended above the ground level. During the trial mesh remained suspended at 3.5 cm above the ground level. The use of the mesh has been previously used by Hedrick and Dill (1993) to explore female crickets' preferences for calling song bout under simulated conditions of risk. Females under low risk conditions females were covered with a folded mesh (Fig. 3). In contrast, absence of cover simulated high risk conditions during trials.

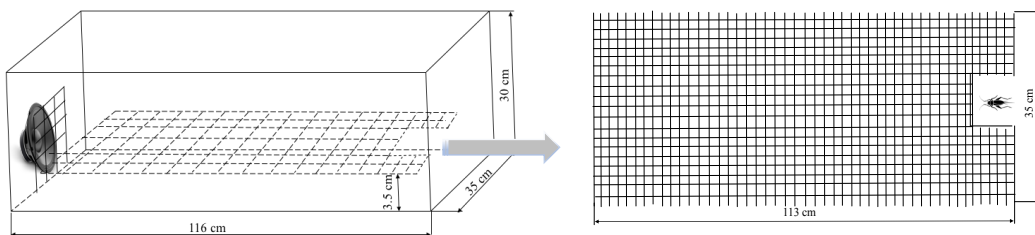


Figure 3. Diagram of experimental arena

A single female under a plastic container was placed at 110 cm from the playback speaker inside the rectangular chamber. Following a 3-minute silent acclimation period, we began playback of a randomly assigned song model (preferred 60% long chirp or non-preferred 20% percent long chirp) in combination with randomly assigned risk levels (high or low). The effect of mesh on the degradation of the sound signal was investigated and ruled out during pilot phase.

The lid covering the cricket was removed right after the playback would start to allow female cricket to respond to the song, which signified the start of the 5-minute phonotactic trial. Immediately after releasing the female, we began a five-minute observation period. The music was played within seconds after female's release. It is typical for females to exhibit phonotactic walking behavior in response to a song stimulus. The criteria for discarding females were if she failed to cross 25-cm line away from the speaker ($n=22$) and if she jumped or flew out of the testing chamber ($n=0$). During each trial, the following variables were measured in response to a broadcast song as a potential proxy for female mate preference: (1) latency, the amount of time it took a cricket to initiate movement (in sec); (2) the amount of time it took a female to reach the line 25 cm from the speaker (in sec); (3) the amount of time it took the female to cross the line 5 cm from the speaker (in sec) (4) the distance at which crickets settle by the end of the 5-minute trial (in cm). Female body mass (measured to nearest mg) and the width of pronotum (measured to the nearest mm), which closely correlates with body mass, were measured and recorded but were not controlled for experimentally.

A total of 266 females were tested. As in other studies, the amount of time crickets spend within 25 cm line from the speaker broadcasting song was determined to

be the choice zone (Atwell & Wagner, 2015). In turn, crickets that never crossed this line in response to playback were considered as non-responders and were excluded from the general analysis. Each female was tested only once with random combination of song rearing, risk conditions (presence or absence of cover) and song model during playback (60% or 20% long chirp). A total of 244 females were tested successfully within the following combination of experimentally controlled variables (Table 1):

Table 1
Trial combinations

		Song Rearing		No Song Rearing	
		Low	High	Low	High
Song model	Risk				
	60% long chirp	N=28	N=30	N=31	N=30
20% long chirp		N=32	N=31	N=32	N=30

Statistics

All statistics were done in R version 099.484. Linear regression was used to test females' response to playback by comparing the amount of time it took a female cricket to reach the line 25 cm and the one 5 cm from the speaker. Both data outputs have been transformed (reciprocal and square root) and tested for normality and for normality of residuals. Binomial logistic regression was used to evaluate females' response to playback by measuring latency in movement and by measuring the distance (cm) at which female crickets settled away from the speaker by the end of the trial. Binomial logistic regression was used because of discrete data distribution in two dependent variables, latency and distance, violations of normality assumptions and normality of residuals associated with it.

The natural break for latency of movement was clustered around 5s, which corresponds to median value. Out of 244 females, 130 responded within less than 5s and 114 took longer than 5s. The natural break for the distance at which crickets settled by end of a 5-minute trial was 2 cm (Fig. 4), which corresponds to the median value. Out of 244 females, 136 settled at 2 cm or less away from the speaker, n=108 settled at more than 2 cm away from the speaker.

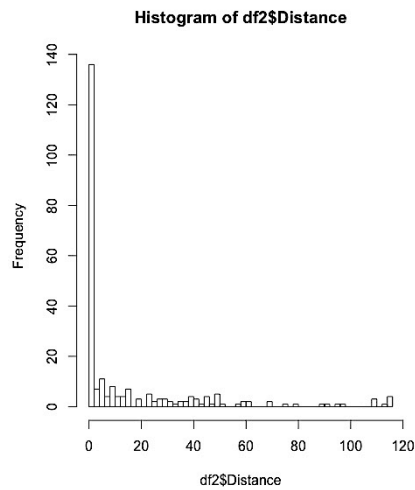


Figure 4. Histogram of the distance at which females settled by the end of the trial

The final model included acoustic rearing, cover conditions, song model, and their interaction as independent variables.

Results

Rearing environment significantly affected the response of females to playback. Consistent with the main predictions, females reared in the No Song environment were more responsive to a song stimulus. It took No Song reared females less time to reach a line 25 cm from the speaker than did females reared in the Song environment, regardless of which song model was used in the playback (F -value=38.24, p <0.01). Similarly,

females reared in the No Song environment took less time to reach a line 5 cm from the speaker than did females housed in Song incubator (F -value=42.25, p <0.01). Females reared in the No Song environment also took less time to initiate movement at the beginning of the trial than did females reared in Song environment (z (240) = 3.16, p <0.01). No other variables or interactions significantly affected the amount of time it took crickets to reach either the 5 or 25-cm line from the speaker. Similarly, no other variable or interaction had a significant effect on the females' latency to begin moving. Of particular interest out of four metrics was the distance at which female crickets settled by the end of the trial. Distance was the dependent variable included in the final model.

Females were significantly more likely to settle less or equal to 2 cm from the speaker if they were reared in the No Song environment (Fig. 5; z (239) = -2.52, p =0.01 (Table 2)).

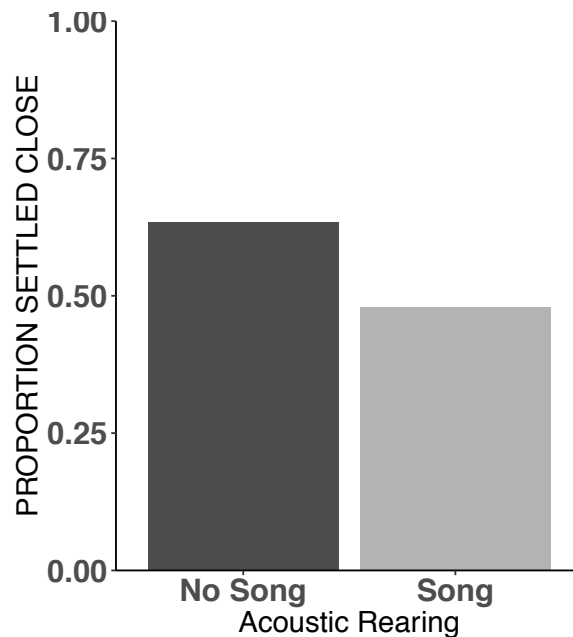


Figure 5. Proportion of females that settled within 2 cm of the speaker in response to playback based on their rearing conditions (No Song=123; Song=121)

This was true regardless of the song model (preferred or nonpreferred) played during the phonotaxis trial (Fig. 6). The influence of acoustic rearing was not significant across song models as evidenced by nonsignificant interaction effect between variables after controlling for risk conditions and was therefore removed from the final model.

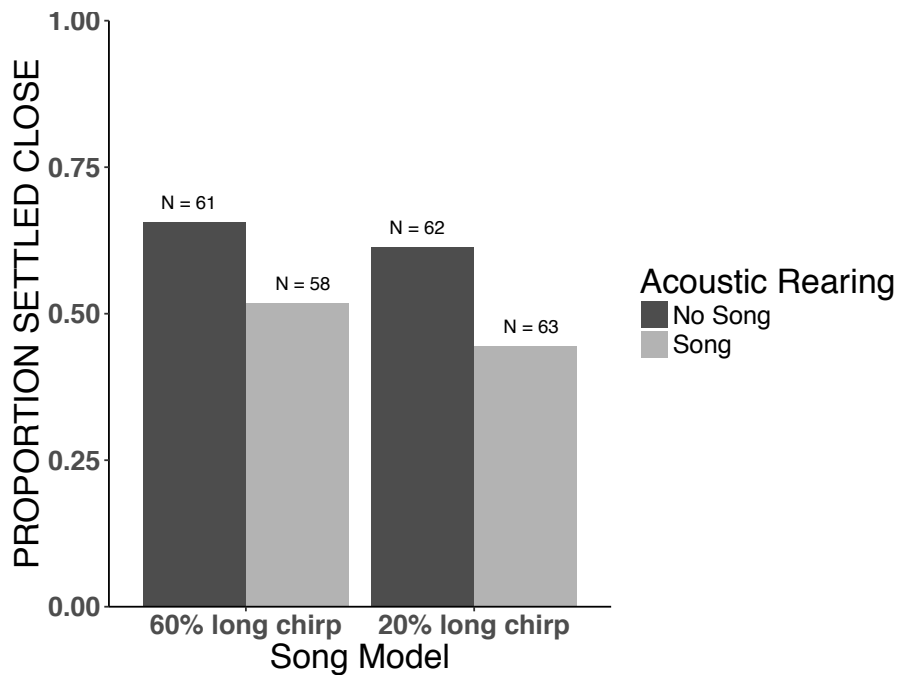


Figure 6. Proportion of females that settled within 2 cm of the speaker in response to playback of songs with either 60% or 20% long chirp

We found a significant interaction between risk conditions and song model indicating that the effect of risk on the distance at which females settled in response to playback was not consistent for all song models ($z_{(239)} = -2.69, p < 0.01$) (Fig. 7). A *post hoc* comparison revealed that the interaction between risk conditions and song model was significant for only one song model, 60 percent chirp song model ($p < 0.01$). Thus, in response to 60 percent long chirp, empirically established preferred song, a higher

proportion of females settled close (2 cm or less) to the speaker under high risk conditions than under low risk conditions (Fig. 7).

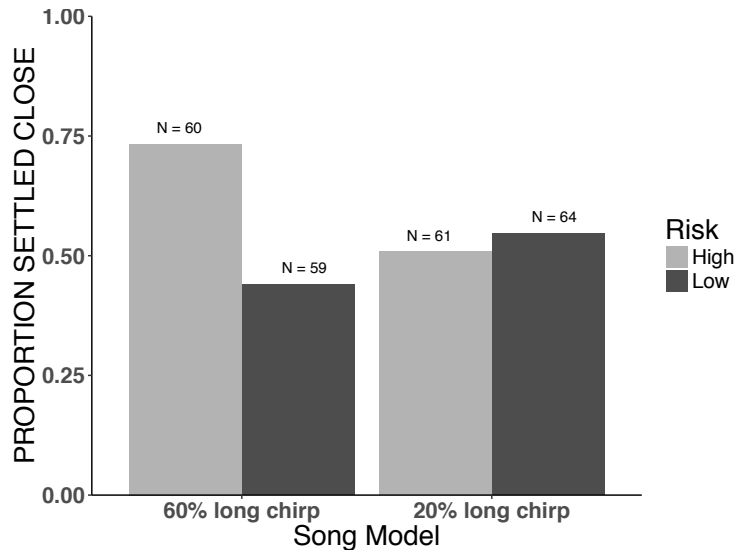


Figure 7. Proportion of female crickets settling close (2 cm or less) to the speaker in response to song models under high and low risk conditions

Three-way interaction between independent variables was statistically insignificant however to test our predictions regarding outcomes within the context of differential acoustic rearing we subset the data. When analyzed separately from the total number of female crickets ($n=244$), crickets reared in No Song environment ($n=123$) demonstrated an interaction between risk conditions and song model ($z_{(119)}=-2.55$, $p=0.01$) (Fig. 8). A *post hoc* comparison revealed that risk conditions particularly matters when female crickets raised on No Song rearing are exposed to 60 percent long chirp ($p=0.03$).

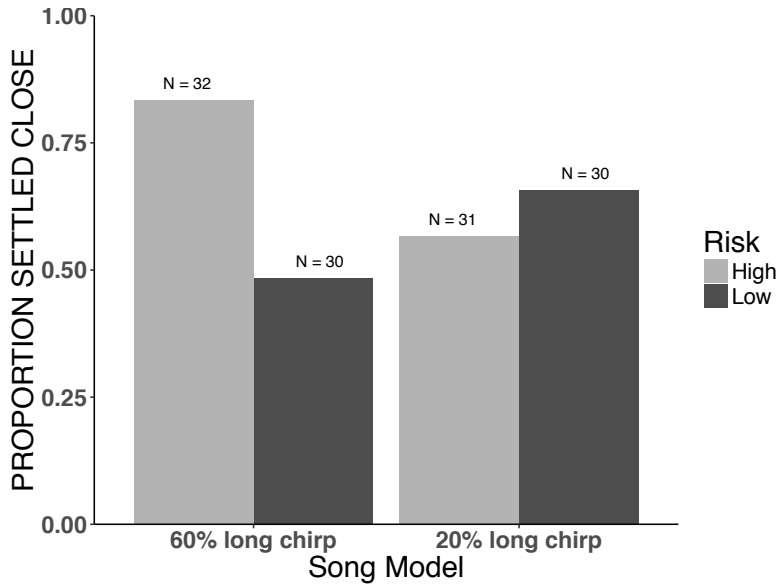


Figure 8. Proportion of female crickets reared in the No Song environment that settled close (2 cm or less) to the speaker in response to song models under high and low risk conditions. Conversely, once analyzed as a subset, females reared in the Song environment (n=121) demonstrated no statistically significant interaction between risk conditions and song model ($z_{(117)} = -1.25, p=0.21$) (Fig. 9).

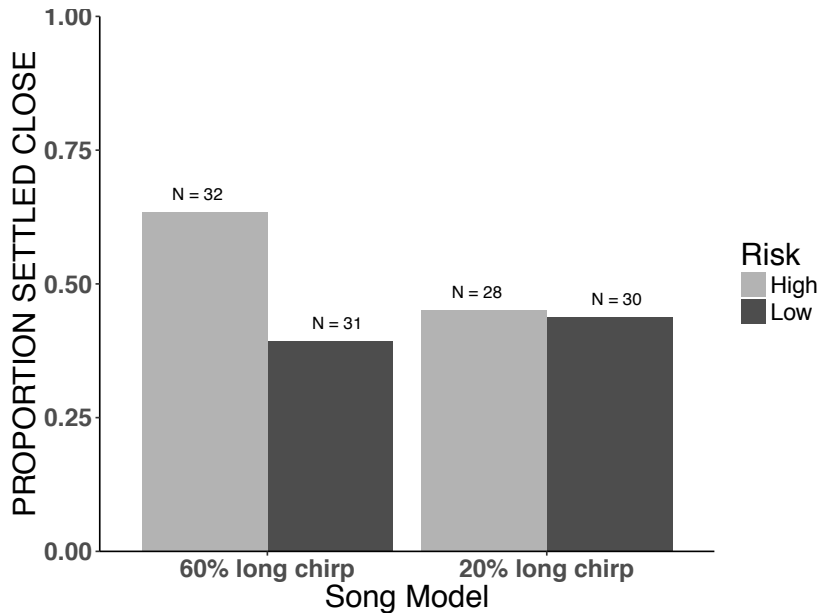


Figure 9. Proportion of female crickets reared in the Song environment that settled close (2 cm or less) to the speaker in response to song models under high and low risk conditions

Table 2

Binomial Logistic Regression Predicting Females that Settled within 2 cm away from Speaker by Acoustic Environment, Song models and Risk Conditions

	Estimate	Z	p
Acoustic Rearing	-0.677	-2.52	0.011*
Song Model	0.999	2.54	0.011*
Risk	0.154	0.42	0.671
Song Model x Risk	-1.453	-2.69	0.007**

Note: Degrees of freedom was 239; N = 244

Discussion

Consistent with prior research (Bailey & Zuk, 2008), our results show higher general responsiveness to playback in females reared in No Song incubator than females housed in Song incubator (Fig. 5). At the same time, our results failed to support our prediction about the significance of the interaction between acoustic rearing and song model, played during phonotaxis trial. Our hypothesis suggested that females reared in Song environment would be more likely to discriminate among song models than females reared in No Song environment. Female crickets in this study, however, did not differ in their response to song models during phonotaxis trials regardless of their acoustic rearing environment (Fig. 6). Thus, female crickets reared in Song environment settled at a relatively comparable distance from the speaker in response to both song models during trials (Fig. 6).

Based on prior empirical findings (Hedrick & Dill, 1993; Pilakouta & Alonzo, 2013), we predicted that the level of risk will affect how close females would get to the speaker playing the preferred song. Female crickets were predicted to settle at a distance far from the speaker playing the preferred song during phonotaxis trials under conditions associated with high risk. In contrast, higher proportion of female crickets settled closer

to the speaker in response to 60 percent long chirp (preferred song model) when under high risk conditions but, were more likely to settle far from the speaker in response to 60 percent long chirp when under low risk conditions (Fig. 7). These results also seem to contrast with theoretical predictions of cost-reducing mating strategy substantiating our hypothesis that females would be more cautious in their mating strategy and less likely to exercise their preferences in the open area associated with high level of risk (Kirkpatrick, 1996; Pomiankowski, 1987; Real, 1990).

Given that sexual selection theory predicts survival costs of mate choice in cases where females exercise preference in the presence of high risk, we consider alternative explanations of our findings. Females may have exercised preferences in the open area (Fig. 7), presumably associated with high risk, because they were exhibiting risk-sensitive behavior (Lafaille et al., 2010). Risk-sensitive behavior reflects trade-off between the cost of fleeing from risk while skipping mating opportunities and the cost of remaining to avoid the loss of mating prospects (Alem & Greenfield, 2010; Lafaille et al., 2010; Ydenberg & Dill, 1986). The amount of actual risk (threat) that animals perceive in given circumstances has been theorized to influence individuals' motivational state and by extension the likelihood of engaging in risky behavior (Helfman, 1989). The extension of this hypothesis is that the actual perception of what is presumed to be risky in lab conditions may be perceived as negligible by biological organisms and influence engagement in presumably risk-sensitive behavior (Alem & Greenfield, 2010). Female crickets may have expressed preferences because, under presumably high risk conditions, the cost of missing a mating opportunity was greater than perception of risk in the absence of actual predator cue during our experiment (Ydenberg & Dill, 1986).

Females, under mesh cover, in presumably low risk conditions, were more likely to settle away from the speaker playing preferred song (Fig. 7), which appears to contrast prior research suggesting that females are more likely to exercise preferences when in low risk conditions (Milinski & Bakker, 1992; Pilakouta & Alonzo, 2013). One way of explaining these outcomes is optimal escape theory, which suggests that distance to a refuge, such as mesh cover, contributes to the assessment of costs associated with exercising preference (Grant & Noakes, 1987; Kramer & Bonenfant, 1987). Therefore, female crickets may be less likely to settle close (2 cm or less) to the speaker playing the preferred song if that meant to be distanced away from the safety of the mesh cover (Kramer & Bonenfant, 1997). This suggestion is empirically supported by Hedrick and Dill (1993) where once in the presence of cover, females tend to limit their time away from it in response to the preferred long calling bout. In the context of our study, females were more likely to settle far (more than 2 cm) from the 60 percent long chirp which allowed them to remain well under cover and in relative proximity to the speaker. For instance, a female that settled at 6 cm from the speaker in response to preferred song model were fully covered by the mesh and still close enough to the speaker. In contrast, females that settled at 2 cm or closer to the speaker would also include individuals that settled immediately next to the speaker, or on the wall without any cover. The significance of remaining fully covered by mesh may explain why females were less likely to exercise preferences, thus limiting their time away from the mesh.

These results are particularly interesting to recognize if considered in the context of plasticity induced by acoustic rearing during juvenile stage and by perception of risk conditions on adult female crickets' response to the playback during phonotaxis trials.

As predicted Song-reared females were less responsive to the song stimulus in general. Contrary to predictions, Song-reared females demonstrated no significant interaction between preference for the song model and risk conditions. Consistent with our predictions, No Song-reared females were predicted to be more responsive to the song stimulus. Although predicted to be more sensitive to risk conditions without discriminating across song models, No Song-reared females settled closer to the speaker in response to the preferred song model under presumably high risk conditions (Fig. 9). These findings may be easier interpreted in the context of challenges that females face in their search for males since the loss of an important sexual signal on the island of Kauai. Higher likelihood of engaging in risk-sensitive behavior appears intuitive since females on the island of Kauai are surrounded by non-calling males and may be more likely to engage in risk to maximize mating prospects. However, higher likelihood of engaging in risk-sensitive behavior exclusively in response to the preferred song model seems to indicate that No Song-reared females expose themselves to risk to pair with the preferred male type which given the real conditions of Kauai appears counter-intuitive. There are not many calling males left on the island of Kauai (Zuk et al., 2006) and the preferred males may not be available to be choosing from. It may be that our results demonstrate partial loss of plasticity in response to acoustic rearing conditions. Females still display plasticity in responsiveness but female preference appears to be inflexible in response to differential acoustic rearing.

In the context of this alternative explanation, it becomes easier to conceptualize why No Song-reared females did not exhibit preference under conditions associated with the presence of mesh or low risk. If these results demonstrate partial loss of plasticity in

female preference, rather than responding to the differential acoustic rearing, female crickets are responding to the immediate presence of mesh. In that case presence of mesh is having a masking effect which obscures female preference for 60 percent long chirp.

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