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Sexual Signal Loss: The Link between Behavior and Rapid Evolutionary Dynamics in a Field Cricket

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Summary

1. Sexual signals may be acquired or lost over evolutionary time, and are tempered in their exaggeration by natural selection.
2. In the Pacific field cricket, *Teleogryllus oceanicus*, a mutation ("flatwing") causing loss of the sexual signal, the song, spread in < 20 generations in two of three Hawaiian islands where the crickets have been introduced. Flatwing (as well as some normal-wing) males behave as satellites, moving towards and settling near calling males to intercept phonotactic females.
3. From 2005-2012, we surveyed crickets and their responses to conspecific song, noting the morph and number of males and females before and after experimental playbacks. The three Hawaiian islands consistently contained different proportions of flatwing crickets, ranging from about 90% of males on Kauai to 50% on Oahu to rare on the Big Island of Hawaii.
4. Flatwing and normal-wing males do not appear to differ in responsiveness to playback, a behavior that should influence the likelihood of a male encountering a phonotactic female. Instead, male and female crickets from populations in which little to no calling song is perceptible during development tended to seek out callers more readily than crickets that developed in noisier environments. Such increased phonotaxis makes females more likely to find either the caller to which they are responding or to encounter a flatwing (or normal male satellite) that has also been attracted to the song.
5. Our evidence suggests that pre-existing behavioral plasticity (manifest as flexible responses to social – particularly acoustic – information in the environment) is associated with the rapid

spread of the flatwing trait. Different social environments select for differential success of flatwing or normal-wing males, which in turn alters the social environment itself.

Introduction

Sexual signals may be acquired or lost over evolutionary time (Wiens 2001), with the likelihood of either determined by how much natural selection imposes a cost on such signals (Fisher 1958; Lande 1981). These losses or elaborations may occur over historical time scales, contributing to diversification (Boughman 2001; Mendelson & Shaw 2005) or more rapidly, over contemporary time scales of a hundred generations or fewer (Hendry *et al.* 2000). Sexual trait loss, as opposed to gain or exaggeration, has been considered relatively little, although it appears to be widespread across taxa and is predicted by theoretical models of sexual selection (Lande 1981; Wiens 2001). Despite the expectation that sexual selection should drive rapid evolution, instances of rapid sexual trait evolution are surprisingly rare, with a systematic literature survey finding only a handful of examples (Svensson & Gosden 2007).

Perhaps the reason for the paucity of examples is that situations that permit sexual signal loss depend on the population dynamics of the organisms in question. If, for example, high population density allows frequent encounters with potential mates, this could compensate for decreased signaling. Alternatively, sexual signal loss could mean fewer natural enemies attracted to the conspicuous signal, which in turn leads to a low mortality rate. Such a decrease in mortality, particularly early in the reproductive period, would increase longevity, and a longer life span would then be expected to compensate for reduced encounter rates (Rotenberry et al. 2015). Because signal loss is rare, however, we lack empirical evidence for the circumstances surrounding signal loss and for its effects on both the signaling sex and the receiver.

Here we address these deficits in our understanding by examining the consequences of rapid sexual signal loss in the Pacific field cricket, *Teleogryllus oceanicus*. *T. oceanicus* occurs throughout a wide range in Australia and Oceania, and has been introduced to the Hawaiian Islands (Zuk *et al.* 1993). In Hawaii, it is attacked by an acoustically-orienting parasitoid fly, *Ormia ochracea*, which finds its hosts by homing in on the male cricket's sexual signal, its calling song (Cade 1975). Crickets and flies occur together on Oahu, Kauai, and the Big Island of Hawaii. The occurrence of cricket populations with and without the fly has enabled us to study how natural selection and sexual selection exerted on the same trait has influenced the signal, and suggested that pressure from the fly drove quantitative changes in calling behavior as well as song structure (Rotenberry *et al.* 1996).

More recently, a new male morph, "flatwing," that lacks the stridulatory apparatus necessary for calling has arisen and spread on two of the islands where the crickets and flies occur, Kauai and Oahu (Zuk *et al.* 2006; Pascoal *et al.* 2014). A third population of crickets on the Big Island of Hawaii has only shown an occasional flatwing. The mutation spread on Kauai within about 20 generations, or 5 years, providing an example of extremely rapid evolution, as well as sexual signal loss (Zuk *et al*. 2006) However, the flatwing phenotype is morphologically distinct and appears to be under independent genetic control in Kauai and Oahu populations, despite sharing the same mode of X-linked Mendelian inheritance (Zuk *et al.* 2006; Tinghitella 2008; Pascoal *et al.* 2014). Flatwings are protected from fly parasitism, but face obvious challenges in mate location and acceptance (Zuk *et al.* 2006; Bailey *et al.* 2008; Tinghitella *et al.* 2009).

This situation affords the opportunity to examine the consequences of the loss of a sexually-selected trait on males and females in a wild setting. Crickets on the two islands with flatwings are now faced with a substantially changed social environment, one that contains far less conspecific song, and far fewer opportunities for encountering a mate, than that found on the Big Island. Laboratory experiments have shown that the acoustic environment influences phonotaxis in both males and females, such that *T. oceanicus* reared without conspecific song respond more readily to playback, behaving more like satellites in the case of males (Bailey *et al.* 2010) and, in the case of females, being less choosy about their response to song variants (Bailey & Zuk 2008). Such an effect of social environment might allow flatwings to more readily encounter females in the field (Zuk *et al.* 2014), and studies using other, closely related grylline crickets have found similar acoustic influences on behavior and life-history traits (Kasumovic *et al.* 2011; DiRienzo *et al.* 2012; Kasumovic *et al.* 2012a, 2012b) . In addition, females from island populations are less stringent in their requirement for males to produce a courtship song before mating (Tinghitella & Zuk 2009). Zuk *et al.* (2014) suggested that pre-existing behavioral flexibility has allowed the mutation to become established despite its sexual selection costs. If crickets already have “rules of thumb” that make satellite behavior, for example, more likely when males mature in a relatively silent environment, whether that environment is silent because of low population density, poor habitat, or the presence of flatwings and hence few callers is immaterial. Since the appearance of large numbers of flatwings on Kauai in 2003, we have conducted regular surveys of crickets and their responses to conspecific song on all three islands in Hawaii, noting the sex, morph, and number of males and females before and after playback (Zuk *et al.* 2006). This long term dataset enables us to examine at a fine scale the population dynamics and behavioral manifestations of a rapidly evolving system in the wild. We were able to capitalize on the fact that the three different Hawaiian islands under study – Kauai, Oahu and Hawaii – have consistently contained different proportions of flatwing crickets, ranging from over 90% on Kauai to approximately 50% on Oahu to barely detectable on the Big Island. The flies are still present on all three islands (unpublished data).

Our purpose here is twofold. First, we document the changes in relative proportions of flatwing and normal-wing crickets since the former first appeared, as well as the responses of both sexes to playback. Second, we tested the hypothesis that differences in response to the acoustic environment have coincided with different evolutionary and population dynamics of flatwing, normal-wing and female crickets on the three islands. Specifically, we address the following questions:

1. How has the abundance of flatwings relative to normal males changed through time on each island? If the ratio of the morphs remains relatively stable on Oahu and Kauai, the acoustic environment is similarly consistent over time, creating the opportunity for selection to act on the novel genotype. Conversely, fluctuations in relative abundance would suggest that the system is unstable, with the potential for local extinction. The contrast between Kauai, which is nearly all flatwing, and Oahu, which has substantial numbers of callers, is particularly informative.
2. How does the relative abundance of flatwing and normal-wing males, reflecting the acoustic environment experienced by each population, influence the responsiveness of females to playbacks? If, as our laboratory experiments suggest, female crickets modify their behavior when they mature in an environment without calling song and become more phonotactic and less discriminating (Bailey & Zuk 2008), we expect phonotaxis to be more pronounced in the more silent environment of Kauai than on the Big Island, with Oahu perhaps intermediate. This response would reflect behavioral plasticity rather than a genetic change in response to selection.
3. How have the responses of both males and females changed over time? *T. oceanicus* breed continuously, with 3-4 generations per year (Otte & Alexander 1974). The flatwing mutation was widespread on Kauai after fewer than 20 generations (Zuk et al. 2006). The survey data reported here span approximately twice that time, which may have afforded the opportunity for selection to have acted on phonotaxis. We were therefore interested in examining whether later responses to playback were different from those at the start of our surveys.

Methods

Field Methods

We conducted cricket surveys and playback experiments on lawns at four sites on three islands: *Hilo*, on the Big Island of Hawaii (University of Hawaii-Hilo campus and First United Protestant Church); *Manoa* (University of Hawaii-Manoa Astronomy Center and Manoa Community Center) and *La'ie* (Brigham Young University-Hawaii) on Oahu; and *Kauai* (Kauai Research Station of the University of Hawaii College of Tropical Agriculture) (Fig. 1). Initial surveys were conducted in July-August 2005, at Hilo, Manoa, and Kauai (Zuk *et al.* 2006); subsequent surveys were conducted opportunistically through November, 2012. Surveys were performed one to two times per year, and included the establishment of La'ie as a site in September 2008 (Table 1).

We delineated 2-m radius circles (survey plots) in areas occupied by crickets (confirmed by seeing or hearing them). We began surveys approximately one hour after sunset, and recorded the ambient temperature at their onset. We collected all crickets within a circle (plot), noting the sex, the wing type, and the number of each, and translocated them away from the circle. Thus we had three types of crickets: females, normal-winged males ("normals"), and flat-winged males ("flatwings"). We then played island-specific calling song for 20 min using speakers placed in the center of the circle. Playback intensity was calibrated to an SPL of 70 dB at 1 m. At the end of the playback period we again collected all crickets inside the circle, noting the sex and wing morphology of each, and measured the distance from each cricket to the speaker. Counts of crickets from the initial removal period reflect the density of each sex and wing type in the occupied area, whereas counts and distances following playbacks are related to a phonotactic response. We deleted from analysis any survey plot in which it rained before the conclusion. To mitigate temperature effects, we also deleted survey plots taken on nights < 20°C (see Appendix 1 for details).

We produced the island-specific calling songs from field-recorded chirps using either Canary v. 1.2.4 or Raven Pro software. Each song contained the mean values of the following components of *T. oceanicus* calling song: pulses per long chirp, long chirp pulse duration, long chirp interpulse interval, short chirp pulse duration, short chirp interpulse interval, short chirps per song, pulses per chirp, intersong interval and frequency for songs recorded in the field at 24–26℃ (see Rotenberry *et al.* (1996) for sonogram). Portions of the survey plot data have been previously reported (Zuk *et al.* 2006, Pascoal *et al.* 2014).

Sampling Adequacy

Collectively, we termed all the nights collecting data at a given site during a given field trip a “site-visit.” We usually sampled about 6 survey plots per night, depending on the weather. We were unable to conduct the same number of survey plots at every site during every visit as both the number of days at a site and their weather conditions varied. Ultimately we deleted as unrepresentative 5 site-visits with n = 3 or 2 survey plots. After incorporating these deletions and those associated with rain or low temperatures, our final data set consisted of 31 site-visit samples with a range of 6-33 survey plots per visit, for a total of 559 survey plots over 103 nights (Table 1). Note that this sampling design is not balanced, which precluded using certain interaction terms in subsequent analyses.

Analysis

For analyses applied to data consisting of either counts or proportions of the number of individuals in a survey plot, we used generalized linear models (GLMs; McCullagh and Nelder 1983) as implemented in SAS PROC GENMOD (SAS Institute 2013) to account for the lack of normality in error terms. For count data we used a log link function and specified a negative binomial error distribution (the latter invariably supplied a better fit than a Poisson distribution); for proportional data we used a logit link and binomial error. Proportional data were analyzed in “events/trials” format (e.g., sex ratio is entered as the number of females and the total number of crickets), where both the number of “events” and the number of “trials” are given as the dependent variables. We evaluated significant differences among main effects based on a Tukey-Kramer test for multiple comparisons. We also assessed correlations of cricket abundances across sites through time.

A complication associated with our data is that in trying to assess correlations of abundances across sites based on individual data points (i.e., survey points), no data point at one site can be specifically linked to one data point in another site, even though sites are sampled at the same time. We adopted a solution provided by Hamlett et al. (2003); for each pair of sites we employed a mixed-effects model (PROC MIXED; SAS Institute 2013) using site as the main (random) effect, month of sampling as a random effect, and survey plots within months as repeated measures. To determine within-site correlations of abundances of cricket types through time we followed a similar approach only with type as the main (random) effect (Hamlett et al. 2003, 2004; Luo et al. 2015). We evaluated significance of correlations in both cases following Luo et al. (2015), who provides a method for estimating confidence limits. For analyses relating to before-and-after counts in response to playbacks we calculated the difference (after minus before, so that a larger value denoted a more positive response) and used a GLM with a normal error distribution. For distances from speakers at end of playbacks, we used a mixed model applied to individuals, treating site and cricket type (and their interaction) as main effects and survey plot as a random effect (PROC MIXED; SAS Institute 2013). Because we had no distances for flatwings at Hilo and no distances for normal males at Kauai (see below) we could not generate least-squares means to statistically compare individual differences among main effects (e.g., differences among cricket types across sites) in the preceding model. Therefore we repeated the analysis omitting site as a main effect but retaining the site by type interaction. All analyses were conducted in SAS 9.3 (SAS Institute 2013). Maps were drawn using R v.2.15.2 (Becker and Wilks 2013a, 2013b).

Results

Patterns of Abundance among Sites and across Time

Flatwings were first noted on Kauai in 2003 (Zuk *et al.* 2006). We performed the first sample point surveys in July/August 2005, and at that time detected flatwings at Manoa as well as Kauai. However, they were about twice as abundant at Kauai than at Manoa (Fig. 2), and they constituted 100% of all males counted in surveys at Kauai, vs. only 40% at Manoa (Fig. 3).

Throughout the > 7 year period, the abundances of each of the three cricket classes varied in different ways among sites (Fig. 2; Table 2). Female numbers, for example, were greater at La'ie and Manoa than the other two sites, and this was paralleled by differences in total crickets. Normal-wing males were absent from all survey plots at Kauai, although we almost always heard a few callers nearby, and flatwings were very scarce in plots at Hilo, whereas the other two sites were intermediate in their abundance of both phenotypes.

Although the numbers of crickets of each type varied substantially among visits to a site, several trends were evident (Fig.2). At La'ie the number of females and normal-wing males significantly increased through time (GLM of number per survey plot vs. month since start of surveys, $p < 0.001$), whereas at Manoa the number of females and normals declined ($p < 0.001$). At Hilo we did not detect the first flatwing until August 2010 (month 62), and flatwings persisted in slightly increasing numbers through the end of our surveys ($p < 0.01$). Females at Hilo also increased ($p < 0.001$), although normal males did not ($p > 0.20$). Females also increased at Kauai ($p < 0.001$). Apart from Hilo, the number of flatwings did not significantly trend through time at the other sites (all $p > 0.20$). Overall, the total number of crickets in survey plots increased significantly at Hilo, La'ie, and Kauai (all $p < 0.01$), and decreased at Manoa ($p < 0.001$). Numbers at Manoa diminished to the point that we no longer surveyed after January 2011.

Flatwings as a proportion of all males sampled differed significantly among sites (GLM of flatwings/total males $p < 0.001$), with Kauai significantly greater than the other sites ($p < 0.001$, Tukey-Kramer comparision), Hilo significantly less ($p < 0.001$), and La'ie and Manoa not differing from each other ($p = 0.56$). Within sites, the proportion of flatwings in samples fluctuated significantly through time at all except Kauai, which was constant (all $p < 0.05$).

Although the number of females varied among sites (Fig. 2, Table 2), the sex ratio as indexed by the proportion of females per survey plot did not differ statistically among sites (GLM of females/total crickets $p = 0.23$; Fig. 4). The sex ratio varied significantly across visits at Hilo, Kauai, and Manoa (GLM, all $p < 0.05$), but not La'ie ($p = 0.91$). In 28 of 31 site-visits females constituted more than half of the crickets counted.

The number of individuals per survey plot in a class sampled at one site was not significantly correlated with numbers sampled at other sites at the same time (r ranged from 0.20 to -0.15, with all 95% confidence limits including zero), although we could not perform all possible comparisons as some mixed models failed to converge to a solution due to too few overlapping site visits (e.g., we visited La'ie and Manoa at the same time on only three occasions). This lack of correlation is consistent with the absence of any apparent synchrony in population fluctuations across sites through time (Fig. 2).

On the other hand, a number of correlations among the abundances of the three types of crickets (females, normal-wing males, and flatwings) within a site were significant (i.e., 95% confidence intervals did not overlap zero). For example, in Hilo, when females were relatively more abundant during a site-visit, so were normal-wing males ($r = 0.37 \pm 0.16$), and flatwings and normal-wings covaried significantly ($r = 0.18 \pm 0.16$). At La'ie, females were correlated with normal-winged and flat-winged males ($r = 0.57 \pm 0.13$ and 0.31 ± 0.15), although the two male types were not correlated with each other ($r = 0.13 \pm 0.18$). On Kauai, where we found no normal-wing males in our samples, the average number of females and flatwing males were significantly correlated (r = 0.31 ± 0.17). At Manoa, only females and normal-winged males covaried significantly (r = 0.63 ±0.13).

Response to playbacks

We assessed cricket response to playbacks of conspecific song in two ways, comparing the number of individuals before and after the playback period, and measuring the distance between each individual and the speaker at the end of the playback period.

Before-after response -- Flatwing response differed significantly among sites (GLM of before/after difference, $p < 0.001$), with Kauai differing from the other sites (greater number of individuals after playback; Tukey-Kramer $p < 0.001$), which did not differ among themselves (all $p > 0.95$) (Fig. 5A). Normal-wing males were somewhat less likely to respond to playbacks than flatwings, and in only a few cases were normals more abundant after playbacks than before. Overall, the response for normal-wing males did not differ among sites (GLM $p = 0.16$; Fig. 5B). Like flatwings, females were significantly attracted to playbacks in several of the samples on Kauai, but not the other sites, and thus differed significantly among sites in their response (GLM $p < 0.001$; Fig.5C). Indeed, they were significantly less abundant in the plots following playback in at least one site visit at Manoa and La'ie, and in three at Hilo.

Differences among the responses of normal-wing males, flatwings, and females were clearly driven by Kauai. All three types co-occur in some abundance at La'ie and Manoa. Pooling those two sites, there is no significant difference in the slopes of the regressions of the number of each type after vs. before playback ($p = 0.12$; Fig. 6). Indeed, the response to playback by flatwings at Manoa and La'ie was virtually the same as that of normal-wing males (Fig. 6 inset). Offsets of the regression lines represent the differences in average abundances of the three types.

Distance Response.--We measured the distance to the speaker for 1841 crickets that responded to our playback experiments (1112 females, 287 normal males, 442 flatwing males). Ninety-eight of 103 nights and 439 of 559 survey plots yielded distances. As before, our design was unbalanced across sites, visits, and nights, with the additional complication that not all cricket types occurred at all sites post-playback.

Average distances for females and normal males did not differ among sites (all Tukey-Kramer $p > 0.5$), whereas those for flatwing males did, with distances at La'ie greater than the other two sites where flatwings occurred ($p = 0.01$; Table 3A). However, over all sites combined, the average distance varied among types ($p < 0.001$), with females and flatwings significantly closer to the speaker than normal males ($p < 0.01$), but not differing from one another ($p = 0.19$; Table 3B). Within a site, patterns differed (Table 3C). At La'ie females approached significantly closer to the speaker than males of either type ($p < 0.05$), which did not differ among themselves ($p > 0.9$), whereas at Manoa females and flatwings were significantly closer than normals ($p = 0.03$; Table 3C). At Hilo, females did not differ from normals, nor did females differ from flatwings at Kauai (both $p > 0.9$)

Overall, the flatwing response to playbacks at Kauai lessened through time: average distance to speaker increased with month since start (mixed model, $p < 0.001$). Otherwise, there were no other statistically significant directional changes of distances through time for any other types at any other sites (all $p > 0.08$).

Discussion

Our surveys of *T. oceanicus* in Hawaii have allowed us to follow population changes in real time during and after a rapid evolutionary event, the spread of a mutation causing the loss of sexual signaling. We first detected flatwings on Kauai in 2003, although they may have occurred at low frequencies before that time (Zuk *et al.* 2006). Although we have consistently found nearly 100%

flatwing males on Kauai over our survey period, we know normal-wing males have never entirely disappeared because we continue to hear a few singing on most visits (Pascoal *et al.* 2014; Zuk *et al.*, unpublished data). The first flatwings on Oahu (at Manoa) were noted in 2005 (Zuk et al. 2006). Here we trace their rise in abundance to approximately 50% of males in survey plots, a proportion that has been relatively stable over time. However, our site on the Big Island has yielded only a handful of flatwings over the years. We do not know why signal loss occurred at different rates on the different islands.

Relative abundance and responsiveness of flatwing and normal-wing males

The acoustic environment on Kauai is essentially silent, and in keeping with our laboratory findings (Bailey & Zuk 2008, Bailey *et al.* 2010), flatwings on Kauai were more responsive to playback than those on Oahu. Normal-wing males on both Oahu and in Hilo were less likely to move in response to playback than were flatwings, perhaps because crickets are generally sedentary signalers and do not travel to find mates (Zuk & Simmons 1997). The responses of the crickets to field playback on all of the islands thus support our suggestion that pre-existing behavioral plasticity was important in the spread of the flatwing morphology (Zuk et al. 2014). In other words, “it’s not only who you are, it’s where you are” – and the acoustic environment in which an individual matures has a fundamental influence on mating behavior. Simply being a flatwing or normal-wing male might not dictate responsiveness to playback, a behavior that should increase the likelihood of a male encountering a phonotactic female. Although this is difficult to test directly in Kauai, where nearly all males are flatwings, on Oahu, behavioral dynamics appear to differ little between the morphs (Fig. 6). Instead, crickets that hear little or no calling song as they mature tend to move toward callers more readily than crickets that develop in an environment with more conspecifics (Bailey & Zuk 2008; Bailey *et al.* 2008; Bailey *et al.* 2010). Such phonotaxis makes a male more likely to behave as a satellite, a behavior that increases the probability of finding a mate (Rotenberry *et al.* 2015).

Coupled with observations about acoustically-mediated flexibility of locomotor behavior in crickets from Kauai but not Hilo (Balenger & Zuk 2015), our results suggest that flexible responses to social – particularly acoustic – information in the environment are associated with the rapid spread of flatwing on Kauai. This behavioral flexibility may have pre-dated the mutation (Bailey *et al.* 2008; Tinghitella *et al.* 2009, Zuk et al. 2014), which would be consistent with such flexibility functioning as a preadaptation promoting the rapid spread of flatwings in Kauai. However, it is challenging to disentangle the idea of a preadaptation from the hypothesis that behavioral reaction norms themselves rapidly evolved during the proliferation of the flatwing phenotype. One possibility is that the behavioral (acoustic) flexibility exists at similar levels in all populations but the current acoustic environment is different on each island owing to the different proportions of flatwing males. Therefore, we see different behaviors in the surveys. Alternatively, the flexibility itself might have differed pre-flatwing such that population reaction norms have different slopes, also leading to the different behaviors we observed. Our observations do not allow us to distinguish among these options, but a combination of laboratory and field work could be fruitfully directed to address this question.

Female abundance, responsiveness, and the acoustic environment

Female crickets were highly responsive to playback on Kauai, but not the other sites. This pattern is consistent with the decreased choosiness and latency to respond to laboratory playback observed in *T. oceanicus* (Bailey & Zuk 2008, Rebar *et al.* 2011), the variation in female response speed induced by prior acoustic manipulations in the congener *T. commodus* (Kasumovic *et al.* 2012a), and decreased female responsiveness in *Gryllus lineaticeps* males with prior experience of higher duty-cycle songs (Wagner *et al.* 2001). Heightened phonotaxis after experience of silence or less attractive songs should increase a female’s likelihood of encountering a male in the field when callers are scarce, either the caller himself or a flatwing that has similarly been attracted to a male’s song. Flatwings still cannot produce a courtship song, the distinct song usually produced once a male and female are in contact, which means that increasing encounter rate is only part of the solution to the flatwings' problem of mate attraction. Indeed, previous work found that while Kauai females are more likely to mate with flatwings than are females from other populations, they still prefer males that can produce a courtship song (Tinghitella *et al.* 2009). Such preferences may help the small number of callers remaining on Kauai persist.

In a laboratory experiment with crickets derived from all three islands, Kauai females reared in a silent environment mimicking a population dominated by flatwings were less responsive to flatwing males in close-range courtship interactions (Bailey & Zuk 2012). Oahu females, however, did not modulate their choosiness depending on their acoustic experience, whereas Hilo females exercised the reverse pattern: they were choosier when reared in silence (Bailey & Zuk 2012). We lack a sufficient sample of populations to make a rigorous statistical comparison, but the apparently consistent relationship recovered in long-term behavioral data from the three islands reported here is striking: behaviors associated with mating responsiveness and phonotaxis are enhanced on the relatively silent island of Kauai, intermediate on Oahu, and relative to Kauai, reversed on Hilo.

When they occurred inside a survey plot, females tended to settle closer to the speaker than did normal males, with no differences in average distance from the speaker among sites. This response is to be expected if the females are approaching a caller to mate, while other males approach callers as satellites following an alternative reproductive tactic (Zuk *et al.* 2014). Normal-wing males settled furthest from the speaker, with flatwing males in between, as would be expected if the normal-wing males are simply benefiting by being in a group while flatwings undertake more risk of aggression by the caller to increase their likelihood of encountering an attracted female.

Changes through time

Cricket abundances covaried among sites differently than they did within sites. The abundance of each class/type of cricket, inferred from pre-playback counts, was unrelated among sites in all cases. Thus, cricket abundances at a given site appeared to fluctuate independent of cricket abundances at the other sites. However, within sites, the abundances of the classes of crickets was more likely to be correlated (6 out of 10 possible comparisons were significant, versus no significant comparisons for among-site comparisons), suggesting that factors affecting the abundance of crickets were site-specific and less likely to reflect larger-scale phenomena, such as climatic variation, affecting all sites in the Hawaiian archipelago.

Over the course of our surveys, flatwings became less responsive to playback. This behavioral trend was particularly noticeable on Kauai, where both flatwing males and females were increasingly non-responsive over successive survey periods, although only significantly so for flatwings. The dynamics on Kauai thus again appear to differ from the other islands, although we do not know whether the trend represents a plastic response to the changing numbers of callers over time, or whether selection has acted on phonotaxis. Although the probability of non-callers, whether flatwing or female, becoming parasitized by flies depositing larvae near the caller is low, it is not zero; a few flatwings, and small percentage of females, have been found to harbor larvae (Zuk *et al.* 1993, N.W. Bailey unpublished data).

The future of flatwings

Our surveys reveal different trajectories for the flatwings on Oahu and Kauai. The different proportions of the flatwings on the two islands acquire new significance given the discovery that the morphs appear to be due to different mutations (Pascoal *et al.* 2014). Because sex determination in crickets is XX-XO, with males always carrying only one copy of the allele, only females can be heterozygous for the trait. We do not know what effects, if any, the allele has in females since they never exhibit wing modifications like those of normal-wing males. It is possible that the allele has pleiotropic effects on males, females or both, and that those effects differ on the two islands, which led to the differing stable proportions of flatwings. Kauai has had a much higher prevalence of fly parasitism than the other islands throughout the 1990s, which may have led to the mutation being favored there first, but this does not explain why substantial numbers of the morph have not proliferated in Hilo on the Big Island , nor why the spread of the mutations was so close in time at the two sites. Intriguingly, the flatwing morphs on Oahu and Kauai are associated with different sets of genetic markers and hence appear to be the result of convergent evolution (Pascoal *et al.* 2014). Mutations causing this and other types of sexual signal loss might thus be more frequent events than previously appreciated, and different genetic causes of sexual trait loss could contribute to variation in the evolutionary dynamics of different populations.

Alternatively, it is possible that some as-yet unidentified ecological factor differs among islands and has influenced the behavioral dynamics and hence the selection pressures. *T. oceanicus* was introduced to Hawaii by humans, but is present in many of the islands across Oceania, where it occurs in disturbed areas such as lawns as well as swampy grasslands (Otte & Alexander 1983, Otte 1994). Although the environment in Hawaii where the crickets occur appears to be roughly the same as that where the species occurs elsewhere, the distribution of *T. oceanicus* within Hawaii is extremely patchy, with many habitats that appear to our eyes as suitable being unoccupied. Furthermore, we know nothing about when or where the fly, which is native to North America, was introduced, and hence the length of time that the cricket and fly have associated on the three islands where they now occur is likewise a mystery. Continued monitoring of the populations, as well as more detailed genetic analyses of the cricket morphs, should shed more light on the future of the novel trait.

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Authors’ Contributions

MZ and JTR conceived the project and developed the methods; all authors collected the data; JTR analyzed the data; MZ, JTR, and NWB led the writing of the manuscript; all authors contributed to revisions and gave final approval.

Data Accessibility

Data used in these analyses (counts of crickets in survey plots and distances from playback speakers) have been placed in the Dryad Digital Repository and are accessible at https:/doi.org/10.5061/dryad.bb384 (Zuk et al. 2018).

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Figure Legends

Figure 1: *Teleogryllus oceanicus* sampling locations in the Hawaiian archipelago (stars). Italicized font indicates population labels used throughout the study.

Figure 2. The number of crickets (average per survey plot over all nights) for each class at each site during each period surveyed. See Table 1 for sample sizes and dates of surveys. Standard error bars omitted for clarity. Note that scale of both axes is the same among panels.

Figure 3. The total number of flatwings as a proportion of the total number of males in a survey plot averaged over all survey plots during each site visit. See Table 1 for sample sizes. Error bars omitted for clarity.

Figure 4. The total number of females as a proportion of the total number of crickets in a survey plot during each site visit. Solid horizontal line denotes 50:50 sex ratio. See Table 1 for sample sizes. Error bars omitted for clarity.

Figure 5. Average number of crickets responding to 20-minute playback of island-specific calling songs by moving into survey plots compared to number removed from survey plots prior to playback. Axes in panels A and B are to the same scale. Data are summarized by site-visit for presentation, although

analyses were based on individual survey plots. Two-way error bars omitted for clarity. Solid line denotes equal numbers after playback as before.

Figure 6. Average number of crickets responding to 20-minute playback of island-specific calling songs by moving into survey plots compared to number removed from survey plots prior to playback, for La’ie and Manoa (both morphs present). In inset, dashed line is linear regression for normal-winged males, solid line for flatwing males. Data are summarized by site-visit for presentation, although analyses were based on individual survey plots. Two-way error bars omitted for clarity.

Table Headings

Table 1. Dates and locations of site visits. Entries are number of survey plots per site-visit used in the analyses.

Table 2. Mean number (and standard deviation) of crickets per sample point per site for each sex and class. Sites had significantly different numbers of crickets of each type ($p < 0.01$) based on a generalized linear model with a negative binomial error term. Values in column with same letter did not differ significantly (Tukey-Kramer test for multiple comparisons).

Table 3. Mean distance (cm) from speaker (and standard deviation) of each cricket type at end of 20-min playback period, and results of mixed effects modeling applied to individuals. Values in column with same letter did not differ significantly (Tukey-Kramer test for multiple comparisons).

Appendix 1. *Temperature Effects*

Temperatures at the start of a night's sampling varied within and across visits, and among sites, from a low of 12°C on 9 February 2012 at Kauai to a high of 30°C on 5 September 2007 at Manoa. Except at the lowest temperatures, activity of crickets (indexed by the average number of all individuals counted per sample plot before or after playbacks in a night) appeared unaffected. Including all 118 nightly samples, temperature accounted for a statistically insignificant 0.4% of the variation in the average number per survey plot before playback (Pearson $r = 0.060$, $p > 0.5$; Appendix Fig. 1). However, cricket movement into survey plots in response to playbacks appeared to slow at the lowest temperatures, yielding a significant correlation between number per survey plot and temperature (Pearson $r = 0.268$, $p < 0.01$; $r^2 = 0.071$). This relationship appeared to be driven by a few samples taken at < 20°C (Appendix Fig. 1); if those are omitted, the correlation becomes non-significant (Pearson $r = 0.177$, $n = 110$, $p = 0.07$) and accounts for only 3.1% of variation in the number of crickets counted after playback. We therefore omitted those survey plots taken on nights < 20°C from further analyses.

Appendix Figure 1. Number of crickets per survey plot per night before and after playback as a function of temperature at the start of sampling. Lines denote linear regressions. Vertical dashed line denotes 20°C threshold for inclusion in subsequent analyses.

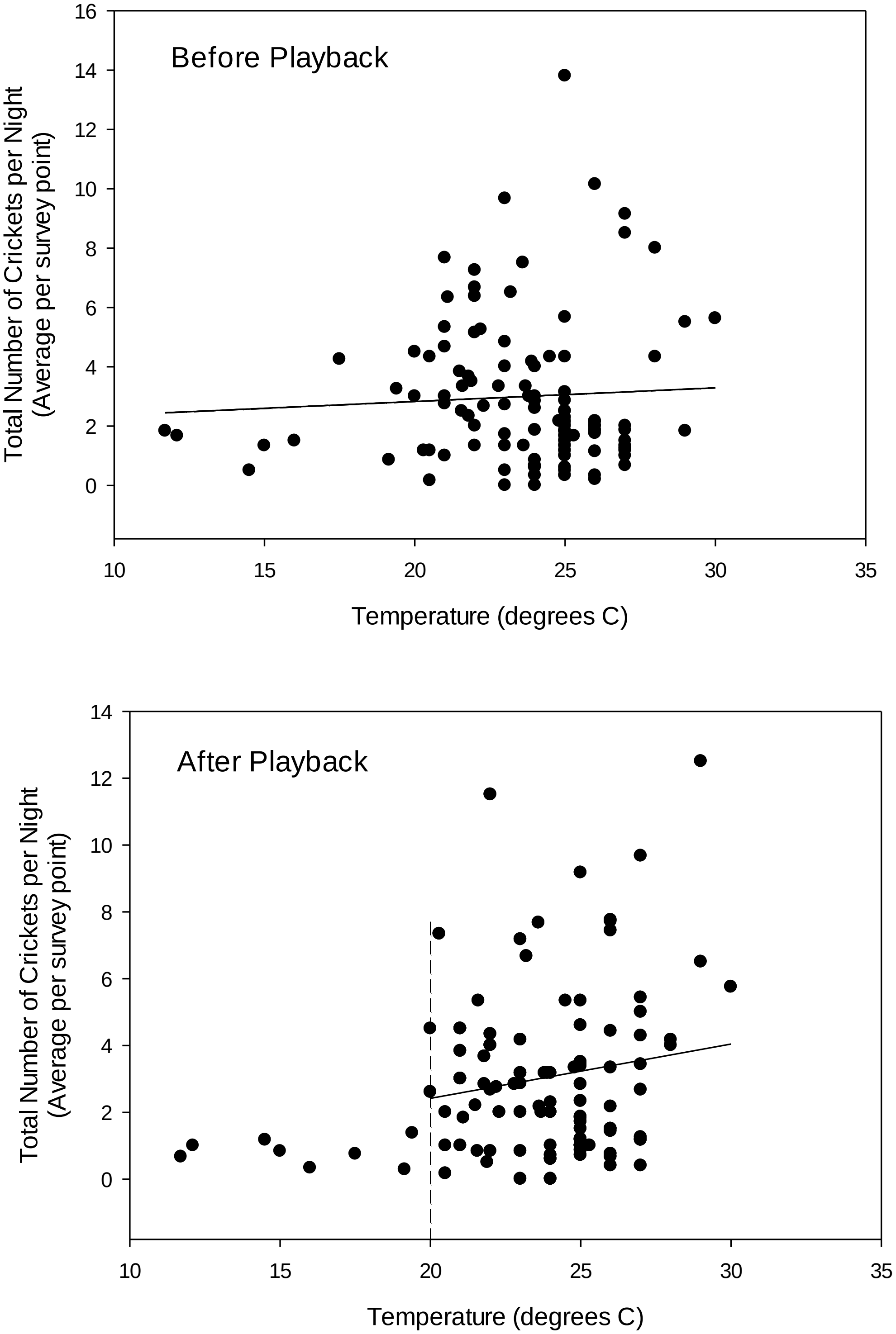


Table 1. Dates and locations of site visits. Entries are number of survey plots per site-visit used in the analyses.

Date of visit	Months since start	Site			
		Big Island	Kauai	Oahu	
		Hilo	Kauai	Manoa	La’ie
25 Jul-14 Aug 2005	0	31	33	22	--
18-22 Apr 2007	21	--	11	10	--
4-17 Sep 2007	26	22	20	24	--
6-20 Feb 2008	31	--	27	24	--
2-11 Sep 2008	38	18	24	18	21
16-22 Mar 2009	44	--	--	--	14
27 Oct-6 Nov 2009	52	11	10	--	19
11-27 Aug 2010	62	20	14	11	12
27 Dec-10 Jan 2011	66	12	--	18	17
12-20 Jun 2011	72	12	--	--	18
7-16 Feb 2012	80	--	6	--	12
14-21 Nov 2012	89	23	--	--	25

-- denotes either site not visited or insufficient sampling (≤ 3 survey plots).

Table 2. Mean number (and standard deviation) of crickets per sample point per site for each sex and class. Sites had significantly different numbers of crickets of each type ($p < 0.01$) based on a generalized linear model with a negative binomial error term. Values in column with same letter did not differ significantly (Tukey-Kramer test for multiple comparisons).

	survey plots	site-visits	Females	Normal Males	Flatwing Males	Total Crickets
Hilo	149	8	1.74 (1.80) b	1.02 (1.35) a	0.05 (0.25) a	2.81 (2.70) a,b
La'ie	138	8	2.60 (2.81) a	0.85 (1.43) a	0.39 (0.75) b	3.84 (4.08) a
Manoa	127	7	2.62 (3.15) a	0.87 (1.64) a	0.46 (0.83) b,c	3.94 (4.64) a
Kauai	145	8	1.54 (1.91) b	0.00 (0.00) b	0.74 (1.15) c	2.29 (2.50) b

Table 3. Mean distance (cm) from speaker (and standard deviation) of each cricket type at end of 20-min playback period, and results of mixed effects modeling applied to individuals. Values in column with same letter did not differ significantly (Tukey-Kramer test for multiple comparisons).

A. Types across sites	Number of Crickets[a]	Females	Normal Males	Flatwing Males
Hilo	160, 109, --	106.8 (61.02) a	115.7 (59.18) a	--
La'ie	320, 100, 48	98.2 (59.93) a	124.1 (53.26) a	126.5 (52.58) a
Manoa	234, 78, 53	95.1 (61.30) a	122.9 (51.61) a	94.5 (60.43) b
Kauai	398, --, 341	94.3 (61.27) a	--	93.6 (62.55) b

B. Among types over all sites	Number of Crickets	Distance
Females	1112	97.4 (60.92) a
Flatwing Males	442	97.3 (62.01) a
Normal Males	287	120.6 (55.10) b

C. Among types within sites	Number of Crickets[b]	Hilo	La'ie	Manoa	Kauai

Females	160, 320, 234, 398	106.8 (61.02) a	98.2 (59.93) a	95.1 (61.30) a	94.3 (61.27) a
Flatwing Males	-- , 48, 53, 341	--	126.5 (52.58) b	94.5 (60.43) a	93.6 (62.55) a
Normal Males	109, 100, 78, --	115.7 (59.18) a	124.1 (53.26) b	122.9 (51.61) b	--

[a] sample sizes listed in order: females, normal males, flatwing males

[b] sample sizes listed in order: Hilo, La'ie, Manoa, Kauai

Figure 1: *Teleogryllus oceanicus* sampling locations in the Hawaiian archipelago (stars). Italicized font indicates population labels used throughout the study.

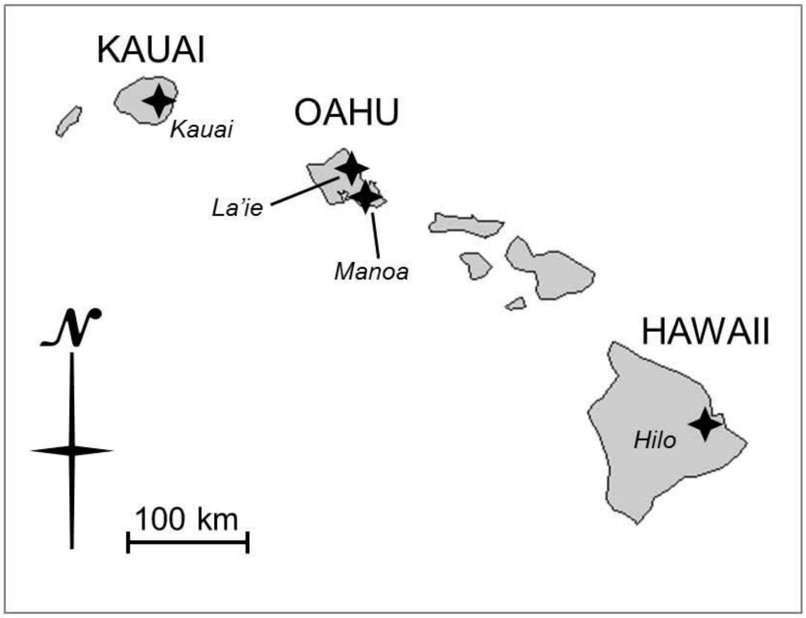


Figure 2. The number of crickets (average per survey plot over all nights) for each class at each site during each period surveyed. See Table 1 for sample sizes and dates of surveys. Standard error bars omitted for clarity. Note that scale of both axes is the same among panels.

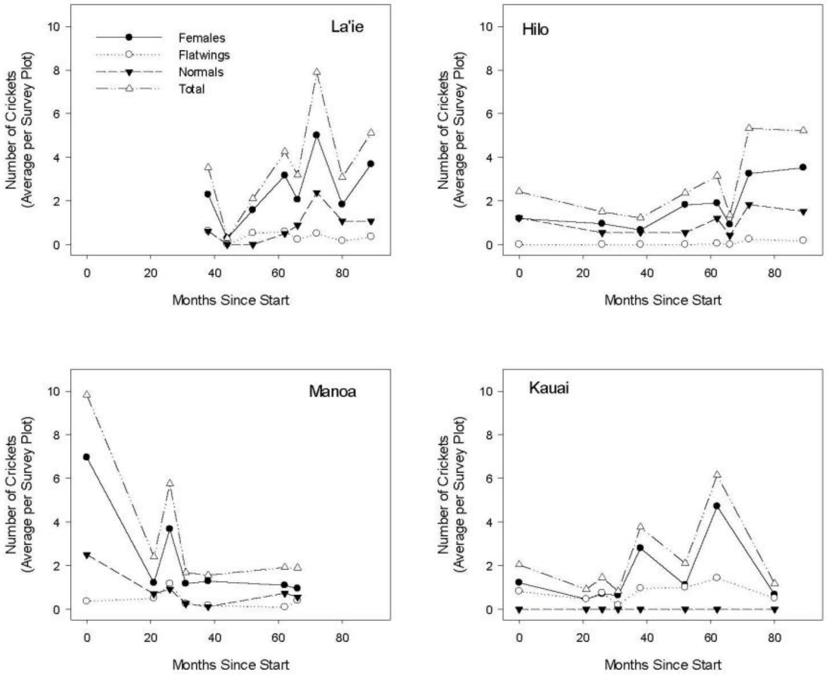


Figure 3. The total number of flatwings as a proportion of the total number of males in a survey plot averaged over all survey plots during each site visit. See Table 1 for sample sizes. Error bars omitted for clarity.

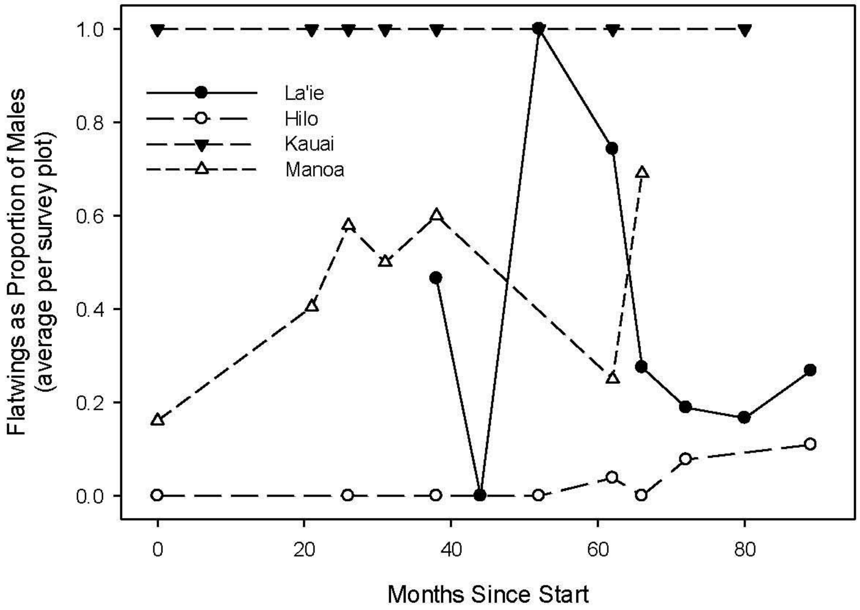


Figure 4. The total number of females as a proportion of the total number of crickets in a survey plot during each site visit. Solid horizontal line denotes 50:50 sex ratio. See Table 1 for sample sizes. Error bars omitted for clarity.

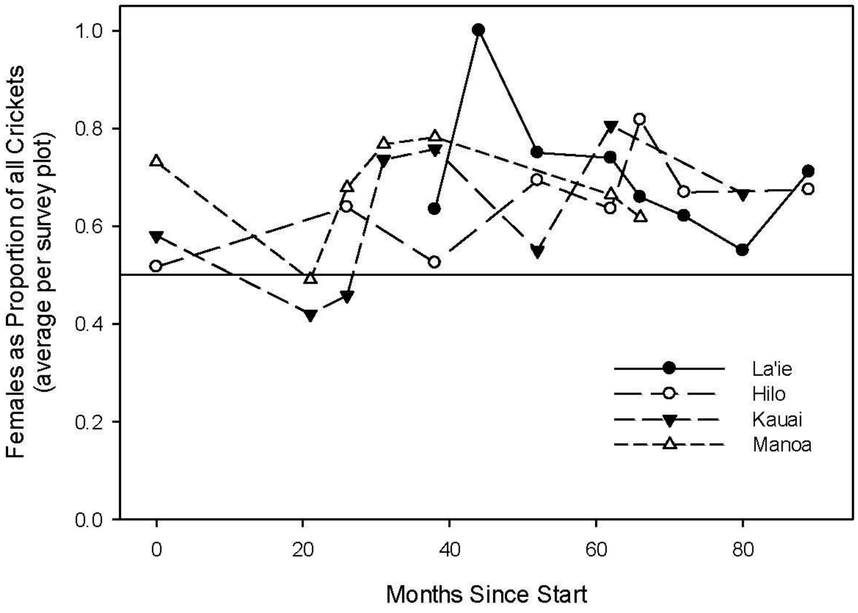
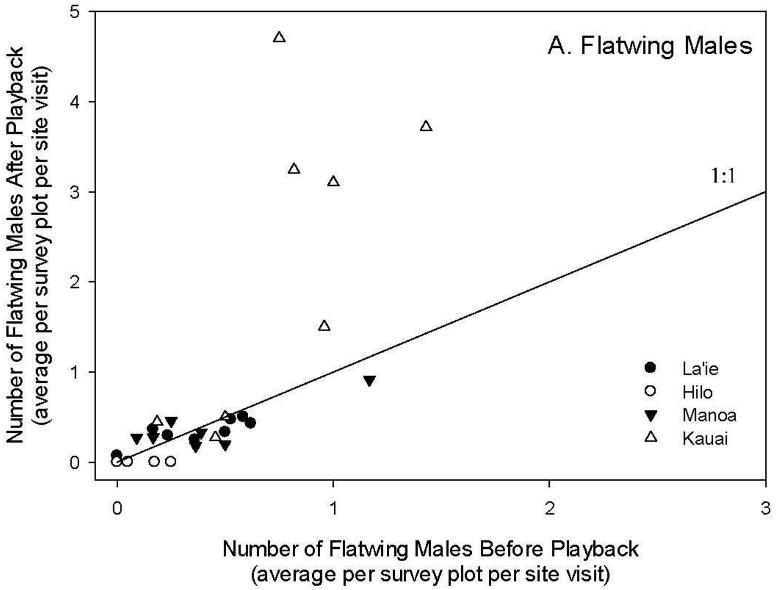
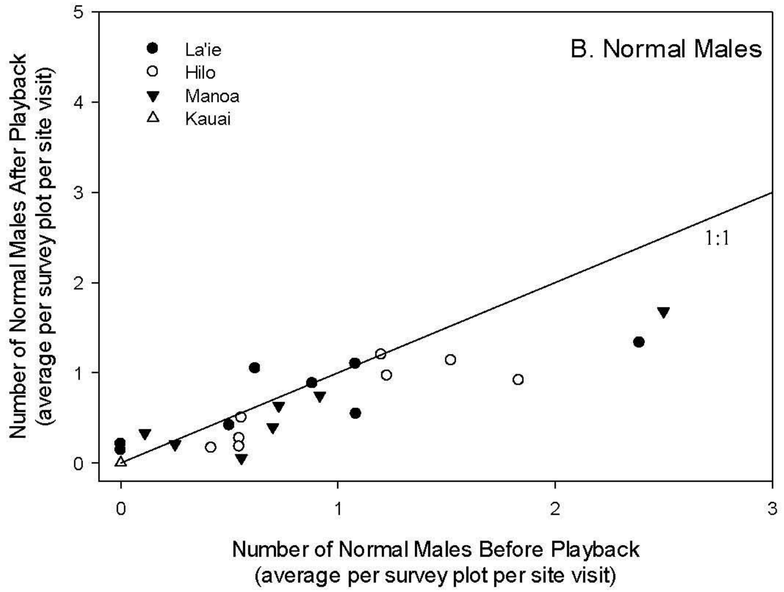


Figure 5. Average number of crickets responding to 20-minute playback of island-specific calling songs by moving into survey plots compared to number removed from survey plots prior to playback. Axes in panels A and B are to the same scale. Data are summarized by site-visit for presentation, although analyses were based on individual survey plots. Two-way error bars omitted for clarity. Solid line denotes equal numbers after playback as before.




B. Normal Males
La'ie
Hilo
Manoa
Kauai
1:1
Number of Normal Males After Playback (average per survey plot per site visit)
0
1
2
3
4
5
Number of Normal Males Before Playback (average per survey plot per site visit)
0
1
2
3

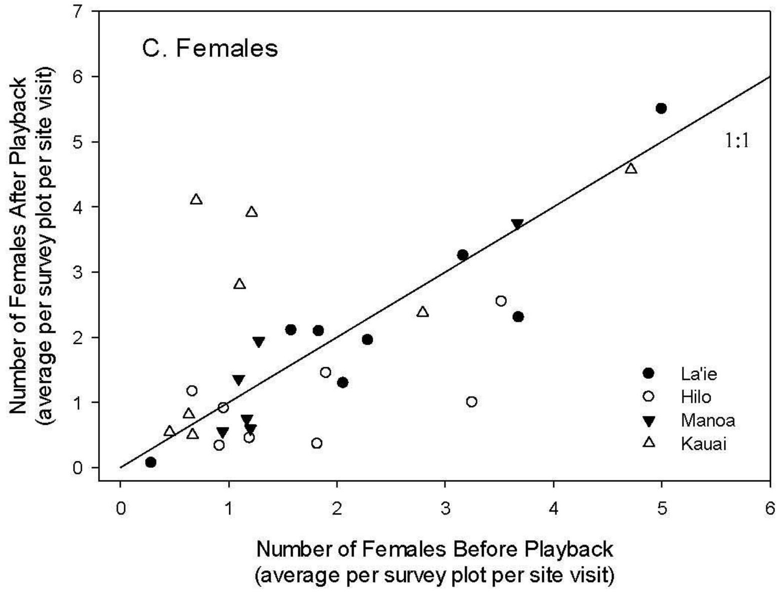

C. Females
La'ie
Hilo
Manoa
Kauai
1:1
Number of Females After Playback (average per survey plot per site visit)
0
1
2
3
4
5
6
7
Number of Females Before Playback (average per survey plot per site visit)
0
1
2
3
4
5
6

Figure 6. Average number of crickets responding to 20-minute playback of island-specific calling songs by moving into survey plots compared to number removed from survey plots prior to playback, for La'ie and Manoa (both morphs present). In inset, dashed line is linear regression for normal-winged males, solid line for flatwing males. Data are summarized by site-visit for presentation, although analyses were based on individual survey plots. Two-way error bars omitted for clarity.