

Direct and indirect effects of sexual signal loss on female reproduction in the Pacific field cricket (*Teleogryllus oceanicus*)

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Abstract

Sexual signal evolution may present fitness consequences for the non-signaling sex due to shared genes and altered social conditions, but this is rarely studied in natural populations. On the Hawaiian Island of Kauai, most male *Teleogryllus oceanicus* (Pacific field crickets) lack the ability to sing because of a novel wing mutation (*flatwing*) that arose and spread in <20 generations. Obligately silent *flatwing* males have been highly successful because they avoid detection by a deadly, acoustically-orienting parasitoid fly. Little is known about how the *flatwing* mutation and resulting song-less acoustic environment affects female fitness. We found that Kauai females carrying the *flatwing* allele invested less in reproductive tissues and experienced more instances of mating failure than *normal-wing*-carrying females, though total offspring production did not differ between female genotypes. Females from Oahu (HI, where the parasitoid and *flatwing* also occur) and Mangaia (an island in the Cook Islands which harbors neither the parasitoid nor *flatwing*) invested less in reproductive tissues when reared in a song-less acoustic environment. Kauai females did not exhibit this plasticity, perhaps because they have experienced nearly song-less conditions for the past ~15 years following the establishment of *flatwing*. We show that female *T. oceanicus* experience a mix of costly and beneficial effects of sexual signal loss, which should help maintain the wing polymorphism in the wild. Our results demonstrate that the non-signaling sex can experience a nuanced set of phenotypic consequences resulting from signal evolution, which can further shape dynamics of sexual signal evolution.

KEYWORDS

female reproductive investment, flatwing, rapid evolution, reproductive success, sexual signal evolution, sexual signal loss, socially-induced plasticity

1 | INTRODUCTION

Understanding the causes and consequences of sexual signal evolution represents a key goal of evolutionary biology. Sexual signals are often expressed in only one sex, typically males, and much research focuses on the drivers of signal diversification

and implications for speciation (Martin, Riesch, Heinen-Kay, & Langerhans, 2014; Miles, Cheng, & Fuxjager, 2017; Panhuis, Butlin, Zuk, & Tregenza, 2001; Safran, Scordato, Symes, Rodriguez, & Mendelson, 2013; Zuk & Simmons, 2018). However, sexual signal evolution can pose fitness consequences for the non-signaling sex, often females, because of shared genes (Berg & Maklakov,

2012; Harano, Okada, Nakayama, Miyatake, & Hosken, 2010; Plesnar-Bielak, Skrzynecka, Miler, & Radwan, 2014; Tarka, Akesson, Hasselquist, & Hansson, 2014) and altered social conditions (Fox, Fromhage, & Jennions, 2019). Alleles underlying male sexual signals can exert negative, beneficial, or neutral effects in females due to pleiotropy or linkage. Because selection tends to favor different trait optima in males and females, intralocus sexual conflict can commonly arise in response to sexual trait evolution (Bonduriansky & Chenoweth, 2009; Connallon & Clark, 2014; Pennell & Morrow, 2013). Female fitness can also be affected by changed social dynamics resulting from signal evolution. These indirect genetic effects (i.e., when an individual's genotype affects trait expression in another individual) can exert novel selection pressures and expose phenotypic plasticity (Bailey, Marie-Orleach, & Moore, 2018; Moore, Brodie, & Wolf, 1997; Wolf, Brodie, Cheverud, Moore, & Wade, 1998). Such direct genetic or indirect environmental consequences of male signal evolution may affect female fitness, which can then have important consequences for evolutionary dynamics in the population (Connallon & Clark, 2014; Fox et al., 2019; Rankin, Dieckmann, & Kokko, 2011).

Pacific field crickets (*Teleogryllus oceanicus*) provide a unique opportunity to investigate the consequences of sexual signal evolution in real time (Svensson & Gosden, 2007). Across their range in Australia and Oceania, *T. oceanicus* males call to attract females (Otte, 1994; Otte & Alexander, 1983; Zuk & Simmons, 1997). In Hawaii, these crickets coexist with an acoustically-orienting parasitoid fly, *Ormia ochracea*, that exploits male calling song to locate its cricket host for larval development which ultimately kills the cricket (Adamo, Robert, & Hoy, 1995; Cade, 1975; Zuk, Simmons, & Cupp, 1993). Female crickets and the deadly fly prefer the same calling song characteristics, generating strong, opposing natural and sexual selection pressures on male song (Rotenberry, Zuk, Simmons, & Hayes, 1996). Between 2001 and 2003, a novel mutation on the X-chromosome that renders males obligately silent arose and quickly spread to ~90% of the males on Kauai (Tinghitella, 2008; Zuk, Rotenberry, & Tinghitella, 2006). Because these silent males lack sound-producing structures on their forewings, we refer to their phenotype as "flatwing." We refer to wild-type males capable of singing as "normal-wing." Remarkably, several years later an independent mutation in a different region of the X-chromosome arose on Oahu that yielded a similar silent (flatwing) male morph, and became established at ~50% of the population (Pascoal et al., 2014; Zuk et al., 2006). After extremely rapid initial spreads on each island, the proportions of flatwing males in Kauai and Oahu have remained stable over the past decade (Zuk, Bailey, Gray, & Rotenberry, 2018). The evolutionary loss of male song has introduced two major changes to affected cricket populations. First, many male and female crickets bear novel alleles associated with the loss of song-producing structures on male forewings that may have pleiotropic effects on other traits (Pascoal et al., 2014; Simmons, Thomas, Gray, & Zuk, 2014; Rayner, Pascoal, & Bailey, 2019). Second, an indirect genetic effect of song loss is that the acoustic environment has rapidly

shifted from song-rich to song-poor, particularly in Kauai, which has drastically altered the perceived social environment (Bailey & Zuk, 2008). These novel alleles and altered social conditions both have the potential to influence female fitness.

Teleogryllus oceanicus possess an XX/XO sex determination system (Hewitt, 1979), where males bear a single X chromosome. The male flatwing morphs on Kauai and Oahu are the result of independent genetic mutations on the X-chromosome that are both inherited as a single locus (Pascoal et al., 2014; Tinghitella, 2008). Though the exact gene and alleles responsible for the two flatwing phenotypes are unknown (Pascoal et al., 2014), we refer to flatwing and normal-wing alleles for simplicity. On each island, males that carry the flatwing allele exhibit the adaptive, silent phenotype that allows them to go undetected by the parasitoid fly (Zuk et al., 2006). Though flatwing males face difficulty attracting mates because they cannot produce the calling or courtship songs that females find attractive (Bailey & Zuk, 2012; Tinghitella & Zuk, 2009), they can achieve matings by adopting alternative mating tactics (Bailey, Gray, & Zuk, 2010; Olzer & Zuk, 2018; Zuk et al., 2006). Briefly, males can act as a satellite to a calling male and intercept females responding to the caller's song. Flatwing males experience other pleiotropic effects of the mutation, including differences in gene expression (Pascoal et al., 2014, 2016), cuticular hydrocarbons (Simmons et al., 2014), and reproductive physiology — flatwing males have smaller testes (Bailey et al., 2010; Rayner et al., 2019) but benefit by siring more offspring per mating event than normal-wings (Heinen-Kay, Urquhart, & Zuk, 2019). Females can be homozygous or heterozygous carriers of flatwing but show no obvious morphological indication of their genotype.

If females experience either detrimental or beneficial pleiotropic effects of flatwing, these can dramatically influence evolutionary dynamics and either hinder or facilitate spread of the silencing allele. Because advantageous X-linked male traits are expressed more frequently due to lack of dominance, deleterious effects on females can be sheltered from selection (Gibson, Chippindale, & Rice, 2002; Patten, 2018; Rice, 1984). If flatwing-carrying *T. oceanicus* females experience reduced fitness from sexual antagonism, the silencing allele could still spread if the benefits for males outweigh the negative impacts on females. Sexual antagonism can also constrain trait evolution because the same allele simultaneously experiences positive and negative selection (Rice, 1984), which could at least partially explain why flatwing has not gone to fixation in Kauai. On the other hand, less sexually dimorphic male morphs are expected to exhibit lower levels of intralocus sexual conflict, and contribute to the maintenance of heritable alternative male reproductive tactics (Plesnar-Bielak et al., 2014). If flatwing has beneficial effects on females, like increased fecundity, or renders females more willing to mate with a flatwing male, the allele would experience positive selection in both sexes, contributing to the rapid spread of the mutation. An additional possibility is that flatwing does not affect female reproduction. Recent work has shown differential gene expression in gonad tissues between flatwing and normal-wing females, but no difference in egg production shortly after reaching sexual maturity

though there appears to be greater variance in egg production of *normal-wing* females (Rayner et al., 2019).

Social cues perceived during development can indicate the abundance of potential mates or competitors an individual is likely to encounter in the future, and can induce plasticity in mating behaviors (Atwell & Wagner, 2014; Bailey & Zuk, 2008; Kasumovic, Hall, & Brooks, 2012). For *T. oceanicus*, rearing in a song-less environment is associated with more permissive female mating behaviors (Bailey & Zuk, 2008, 2012; Lierheimer & Tinghitella, 2017; Swanger & Zuk, 2015), and male behaviors that should enhance alternative mating tactics, particularly in crickets from Kauai (Bailey et al., 2010; Balenger & Zuk, 2015). Such socially-induced behavioral flexibility likely represents a key factor underlying how *flatwing* became established (Zuk, Bastiaans, Langkilde, & Swanger, 2014; Zuk & Tinghitella, 2008). Social exposure to sexual signals is also known to influence reproductive physiology in male crickets. Male *T. oceanicus* raised in the absence of calling song, mimicking a population with high density of *flatwing* males or few competitors, invested less in testes size (Bailey et al., 2010) and exhibit lower sperm viability (Gray & Simmons, 2013), although acoustic experience does not appear to affect male reproductive success (Heinen-Kay et al., 2019). Most studies have found that female crickets reared in the absence of song show reduced investment in reproductive tissues (Bateman, Verburgt, & Ferguson, 2005; Conroy & Roff, 2018; Kasumovic, Chen, & Wilkins, 2016; Kasumovic, Hall, Try, & Brooks, 2011; Rebar, Barbosa, & Greenfield, 2019), similar to males, while one study showed no effect of acoustic experience on egg laying (Lierheimer & Tinghitella, 2017). If experiencing a song-less environment during rearing is associated with lower reproductive outcomes in *T. oceanicus* females, sexual signal loss could create a "tragedy of the commons" in which the very trait that benefits males (obligate silence) has negative effects on population fitness through reduced female fecundity (Rankin et al., 2011).

The goal of this study is to understand how the direct (genetic) and indirect (acoustic environment) effects of sexual signal loss influence female reproduction in *T. oceanicus*. First, we investigated whether female investment in reproductive tissues, mating behavior, and offspring production are associated with the *flatwing* allele in the Kauai population. To accomplish this, we constructed outbred single allele colony lines that breed true for male wing morph, ensuring that females are homozygous for the allele associated with each line. These colonies provide a tractable way to investigate how the genetics underlying sexual signal evolution influence the non-signaling sex without requiring molecular assays or controlled breeding. If *flatwing* females have better mating outcomes than *normal-wing* females, it could help explain the rapid success of the silencing mutation. On the other hand, if the *flatwing* allele is associated with lower reproductive outcomes for females, it could help explain why the mutation has not swept to fixation in Kauai, where ~90% of adult males in the wild exhibit the *flatwing* phenotype but callers still remain at low frequency (Zuk et al., 2018). Second, we examined indirect genetic effects of sexual signal loss on female investment in reproductive tissues by exposing female *T. oceanicus* to either a

song-rich (mimicking an ancestral population with many *normal-wing* males) or song-less environment (which could occur due to either a lack of males, or the presence of many *flatwing* males) during late juvenile stages and early adulthood. We focused on three populations that differ in proportion of *flatwing* males in the wild: Kauai (~90% *flatwings*), Oahu (~50% *flatwings*), and Mangaia, an island in the Cook Islands where neither the parasitoid fly nor *flatwing* phenotype occurs. Examining females from populations with different evolutionary histories will provide insight about whether physiological responses represent a general, species-level response to social cues or a unique response in populations that harbor *flatwing*.

2 | MATERIALS AND METHODS

2.1 | Cricket colony construction and maintenance

Laboratory colonies of *T. oceanicus* were reared in Caron Insect Growth Chambers (model 6025) that maintain a 26°C, 75% humidity environment and photo-reversed 12:12 light-dark cycle. Crickets were housed within 15L plastic containers with ad lib access to Teklad high-fiber rabbit chow, moist cotton for water and oviposition, and egg carton for shelter. Cricket boxes are cleaned at least twice per week. The Kauai laboratory colony was established in 2003 after the discovery of *flatwing*, and the Oahu colony was established in 1993. Both Hawaiian colonies have been supplemented with eggs from wild females at least annually. The Mangaia colony was established in 2009. Laboratory colonies consist of approximately 100 adults at any given time. *Teloegryllus oceanicus* breed year-round and mate multiply (Tanner, Swanger, & Zuk, 2019). Females are sexually receptive as early as 4–5 days past adult eclosion (Bailey & Zuk, 2008). In Hawaii, females have been documented to live up to 17 days past eclosion in the field (Simmons & Zuk, 1994) and are capable of laying eggs throughout this time (Loher & Edson, 1973).

We constructed pure-breeding *normal-wing* and *flatwing* colonies derived from our general Kauai laboratory stock. Juvenile females were removed from the general Kauai colony as soon as an ovipositor was visible, and placed in 15L single sex boxes until adult eclosion. Upon eclosion, individual virgin females were placed in an 118 ml plastic container with food, water, and egg carton. When females were 5–8 days old, a single *normal-wing* or *flatwing* male from the general Kauai colony was added to the container where they remained together for three days. Then the male was returned to the general Kauai colony, and the female was placed in a 1.8L container with moist cotton for oviposition. Females were allowed to oviposit for two days, then were returned to the general Kauai colony. Hatchlings from these matings were reared in sibling groups until adulthood. We used F1 male phenotype in combination with parental male phenotype to determine the parental female genotype. If the F1 generation included at least 10 males that all matched the parental male wing morph, then F1 females were assumed to be homozygous for the associated genotype. If F1 males all expressed the same phenotype, but did not match the parental male, then the F1 females were assumed to be heterozygous. If F1 males showed a

mixture of the two phenotypes, then F1 females were assumed to be a mixture of homozygotes and heterozygotes. A total of 58 females were paired with normal-wing males and 59 females were paired with flatwing males. Ultimately, we identified 5 pairings that resulted in homozygous *normal-wing* offspring and 5 pairings that resulted in homozygous *flatwing* offspring. Although most Kauai males in the wild exhibit the *flatwing* morphology, there are far fewer *flatwing* males in the lab colony. We believe this is because wild Kauai females find and preferentially mate with the few *normal-wing* males still present in the population, as was recently documented in the Oahu population (Tanner et al., 2019). Most of the laboratory females used in our matings were heterozygous, contributing to the relatively low proportion of matings that yielded homozygous offspring.

8–25 homozygous F1 female offspring per pairing were mated with a single male with the corresponding wing morph from the general Kauai colony. F2 males and females were separated as soon as sex differences were evident. Once F2 males were confirmed to exhibit the predicted phenotype, we combined lines to generate the pure-breeding colonies. A subset of 25 F2 females from each pairing were haphazardly removed and split equally amongst breeding boxes (i.e., 5 females from each of five families in five *normal-wing* boxes, for a total of 125 breeding females for each wing morph). All F2 males were sacrificed by freezing to reduce potential for inbreeding. Twenty-five adult males were isolated from the general Kauai colony prior to eclosion and added to each female box with corresponding wing morph. Males and females were haphazardly mixed across boxes within each wing morph at least once per week to maximize potential for outbreeding. Every 2 weeks, a subset of males was removed and replaced with new males with the appropriate wing morph from the general Kauai colony. Upon hatching, juveniles within each breeding group were combined. For subsequent generations, the *normal-wing* and *flatwing* colonies were maintained in the same way as the general laboratory colonies, except that adult males from the general Kauai lab colony were added to the appropriate wing morph colony every several months to maintain genetic variation and ensure that the *normal-wing* and *flatwing* colonies reflect the wild Kauai population. Both colonies have bred true for wing morph since 2016.

2.2 | Direct genetic effects of sexual signal loss

2.2.1 | Reproductive tissue investment

We maintained late instar juvenile females from the pure-breeding *normal-wing* and *flatwing* colonies in separate 5.7L boxes and checked them daily for adult eclosion. Upon eclosion, adult females were transferred to an individual 118 ml rearing container with food, water and egg carton, but no oviposition substrate. Juvenile boxes and individual containers were housed in an incubator containing *normal-wing* males, mimicking conditions when *flatwing* first arose. Females were freeze-killed 8–12 days post-eclosion, then dissected the following day. We measured wet weight of the whole, intact body to the nearest 0.01 mg using a Sartorius balance. We then carefully removed female reproductive tissues (i.e., eggs and ovarioles) and measured

their wet weight to the nearest 0.01 mg. We dissected 32 *normal-wing* and 34 *flatwing* females to obtain estimates of female reproductive investment. Reproductive tissue mass relative to body size is a commonly used metric of reproductive investment (Zuk, Simmons, Rotenberry, & Stoehr, 2004). Because females can produce eggs over the course of their lifetime (Loher & Edson, 1973), this provides a snap-shot estimate of female reproductive investment.

Log-transformed reproductive tissue weight was significantly associated with log-transformed soma weight (mass of the whole body – reproductive tissues; $F_{1,64} = 4.947$; $p = .030$). We performed a general linear model with log reproductive tissue weight as the response, and genotype, age and log soma mass as predictors, and all relevant interactions (Tomkins & Simmons, 2002). Neither interaction term yielded p -values < 0.1 , so we removed them from the final analysis (Engqvist, 2005). All data were analyzed in JMP Pro 13 unless otherwise noted.

2.2.2 | Mating behavior and reproductive success

Because potential reproductive capacity may not reflect actual reproductive outcomes, we tested whether female genotype is associated with the number of offspring produced from a mating. We reared juvenile *normal-wing* and *flatwing* females and *flatwing* males in separate boxes. We used *flatwing* males because they are most abundant in the wild Kauai population, and it allowed us to more easily control female perception of male attractiveness as mediated through song quality. As described above, we checked boxes daily for eclosions and isolated adults in individual rearing containers. At 6–13 days past eclosion, virgin females were paired with a *flatwing* male at least 6 days post-eclosion. Each male was allowed only one successful mating event to maintain independence of female reproductive outcomes.

Matings took place during the crickets' normal active period (09:00 – 21:00) in a 12 cm × 17 cm plastic container with 10 cm high walls in a 21.4–23.7°C anechoic room under red light. We placed a fresh piece of paper towel on the mating arena floor to reduce accumulation of chemical cues. After placing a haphazardly chosen male and female into the mating arena, we began playing a continuous loop of courtship song at 65–70 db SLP (measured 10 cm from the speaker with an AZ sound level meter model 8922) through a mesh-covered cutout of the mating arena wall. Females were presented with a standard courtship song because they are less likely to initiate mounting in the absence of courtship song (M. V. Kota, E. M. Urquhart, & M. Zuk, unpublished data; Tinghitella & Zuk, 2009). We recorded female latency to mount the male after the onset of courtship song, a commonly used metric of female preference in crickets (Shackleton, Jennions, & Hunt, 2005). Once the female mounted the male, we stopped the courtship song. If mating failed to occur within 5 min, we terminated the trial. Each cricket was allowed no more than three mating attempts (one per day) before exclusion from the experiment. Following spermatophore transfer, we gently coaxed the female into a 118 ml container and placed it in a quiet, dark space. We used forceps to remove the spermatophore 30 min after the pair uncoupled to control for the amount of sperm transferred, as sperm

transfers linearly with time (Simmons, Wernham, Garcia-Gonzalez, & Kamien, 2003). Females were discarded if the spermatophore was prematurely removed.

The female was then transferred to an individual 11 cm × 11 cm container with 6 cm high walls with rabbit chow, egg carton, and moist cotton for oviposition. Females had the opportunity to oviposit for 1 week, then were freeze-killed. Female pronotum width was measured to the nearest 0.01 mm using digital calipers. At least five times per week, we checked each cotton container for hatchlings. If present, hatchlings were freeze-killed, counted, then removed from the container. Cotton was disposed of when no hatchlings emerged for at least three days.

Of 129 mating trials, mounting failed to occur in 48 cases (37% of trials), four females died during the oviposition period, and there was one case of a spermatophore breaking. To test whether female genotype was associated with mounting success, we employed a generalized linear mixed model with binomial distribution in the lme4 package of R v.3.4.4 (R Core Team, 2018); male and female IDs were included as random effects because some individuals attempted multiple matings. For females that received a spermatophore, we used a general linear model to test whether log₁₀-transformed female latency to mount differed between *normal-wing* and *flatwing* females. We initially included female age and male and female pronotum widths, but removed them due to non-significance (all *p*-values >0.1). Of 77 females that successfully mated and had the opportunity to oviposit for one week, 67 produced offspring (36 *normal-wing*, 31 *flatwing* females). We used a logistic regression to test whether female genotype was associated with mating failure (i.e., when an apparently successful mating does not yield offspring). Among females that produced at least one hatchling, we used a general linear model to test whether the number of offspring produced was associated with female genotype; female pronotum width and age were included as covariates.

2.3 | Effects of social environment on reproductive investment

Juvenile female crickets from the Kauai, Oahu, and Mangaia laboratory colonies were isolated in 118 ml containers at least two weeks prior to adult eclosion when the auditory structures become developed. Crickets were randomly assigned to either “song” or “no song” acoustic rearing treatment. Both the “song” and “no song” experimental incubators were lined with sound-absorbing foam and equipped with six Sony SRS M30 speakers and three Sony CD players. In only the “song” incubator, a typical calling song from each population was broadcast during the cricket's active period at 80–85db SPL measured at the lid of the cup (Bailey et al., 2010). Populations were tested sequentially, so females were only exposed to calling song from their population of origin. Acoustic treatments and corresponding crickets were periodically swapped to avoid potential for incubator effects. Between 8–12 days past eclosion, virgin females were freeze-killed, weighed, then dissected for reproductive tissues as described above. We excluded seven individuals from the dataset that did not possess any developed eggs (Kauai “no

song” = 4, Kauai “song” = 2, Oahu “song” = 1). Final sample sizes are as follows: Mangaia “song” = 49, “no song” = 50; Oahu “song” = 64, “no song” = 66, Kauai “song” = 28, “no song” = 31.

We used a general linear model to test for differences in female reproductive investment as an indirect consequence of sexual signal loss. Log-transformed reproductive tissue mass served as the dependent variable. Acoustic treatment, population, and their interaction served as fixed factors, and age and log-transformed soma mass were included as covariates. We initially included all relevant interactions between fixed factors and covariates; however, no interactions involving covariates yielded *p*-values <0.1, so we removed them from the analysis. We employed Tukey HSD to disentangle group differences.

3 | RESULTS

3.1 | Female genotype

3.1.1 | Reproductive investment

Log-transformed female reproductive tissue mass was significantly associated with genotype, log-transformed soma mass, and age (Table 1). After controlling for soma mass and age, *normal-wing* females from Kauai possessed ~15% heavier reproductive tissues than *flatwing* females (Figure 1a). Reproductive tissue mass significantly increased with both soma mass and age (Table 1).

3.1.2 | Mating behavior and reproductive success

Normal-wing females were somewhat less likely than *flatwing* females to mount a *flatwing* male, though this trend was not statistically significant (*p* = .0833; 31 *normal-wing* and 17 *flatwing* females did not mount). Among females that did mate, the latency to mount a *flatwing* male did not differ between female genotypes ($F_{1,79} = 0.170$; *p* = .681); *flatwing* females took 45 ± 5 (mean \pm SE) seconds to mount, and *normal-wing* females took 48 ± 5 s. *Flatwing* females were more likely to experience mating failure than *normal-wing* females ($\chi^2_1 = 7.618$; *p* = .006; 9 of 10 females). Although *flatwing* females possessed less reproductive tissue than *normal-wings*, the number of offspring produced did not differ between female genotypes ($F_{1,62} = 0.838$; *p* = .364; Figure 1b). Female age was not a significant predictor of offspring production ($F_{1,62} = 0.164$; *p* = .687; slope = 3.161). Female pronotum width ($F_{1,62} = 8.944$; *p* = .004) was positively associated with offspring production.

TABLE 1 General linear model results examining differences in log-transformed reproductive tissue mass between *normal-wing* and *flatwing* virgin female *Teleogryllus oceanicus* descended from Kauai

Effect	F	df	p
Genotype	5.7784	1, 62	.0192
Log soma mass	17.2170	1, 62	.0001
Age	19.6953	1, 62	<.0001

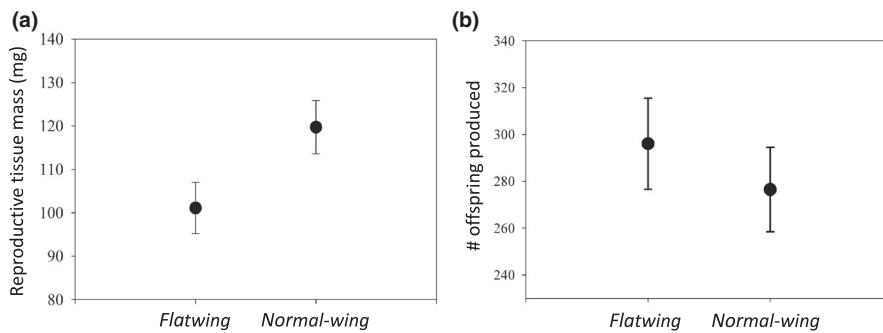


FIGURE 1 (a) Virgin female *Teleogryllus oceanicus* females from Kauai carrying the *flatwing* allele possessed significantly lower reproductive tissue mass (eggs and ovarioles) than *normal-wing* females after controlling for soma mass and age. (b) Female genotype was not associated with reproductive success when mated with a *flatwing* male. LS means \pm SE depicted

3.2 | Acoustic environment

Acoustic treatment, but not population of origin, was significantly associated with log-transformed reproductive tissue mass (Table 2). Female *T. oceanicus* raised in a song-rich environment invested more in reproductive tissues after controlling for body size and age than those raised in the absence of song (Figure 2). However, acoustic treatment and population showed a significant interaction, demonstrating that the greater reproductive investment in song-rich conditions was largely driven by Mangaia and Oahu (Table 2, Figure 2). Tukey HSD revealed no difference in reproductive tissue investment between Mangaia and Oahu females raised in the presence of song, and that Kauai and Oahu females raised with song exposure exhibit similar levels of investment. The Tukey test further revealed that reproductive investment of Kauai females did not differ between acoustic environments, and that both groups of Kauai females are indistinguishable from Mangaia and Oahu females raised in a song-less environment (Figure 2). Log-transformed reproductive tissue mass increased with both log-transformed soma mass and age (Table 2).

4 | DISCUSSION

Our study of the direct (genetic) and indirect (social environment) effects of sexual signal loss on female reproduction provides a rare examination of how the non-signaling sex is affected by contemporary sexual signal evolution in the wild. *Flatwing* males in Kauai simultaneously experience costs (incapable of singing to attract mates) and benefits (undetected by the deadly fly, sire more offspring per successful mating event) directly associated with the phenotype generated by the silencing mutation. We found that, similar to males, the consequences of signal loss for females presents neither a clear cost nor benefit, but rather a mixed set of effects that differs between populations. This is an important contribution because it demonstrates that females do not always experience unambiguous fitness costs or benefits owing to male sexual signal evolution or heritable alternative reproductive tactics (e.g., Harano et al., 2010; Plesnar-Bielak et al., 2014). We suggest that these nuanced consequences of signal loss help maintain the wing polymorphism in Hawaii.

We discovered differences in reproductive tissue between *flatwing* and *normal-wing* females, but not in the number of offspring

produced. In our snap-shot estimates of reproductive investment, the reproductive tissue of *flatwing* females from Kauai weighed considerably less than those of their *normal-wing* counterparts, though *flatwing* and *normal-wing* females ultimately achieved the same reproductive success. The *flatwing* allele should confer an advantage to females if they are able to divert this resource savings to other areas, such as immunity or maintenance. However, it is unclear whether this resource efficiency ultimately provides a fitness benefit or reflects a tradeoff with other aspects of reproduction. For instance, *flatwing* females may possess the same number of eggs as *normal-wing* females, but these eggs may be smaller and poorer quality. Crickets are capable of facultative shifts in egg laying schedules (Adamo, 1999), and exhibit a great deal of variance in the number of eggs stored vs. eggs laid at any given time (Loher & Edson, 1973). The balance of egg use may differ between female genotypes. In another species of Gryllid, differences in reproductive schedule are genetically correlated with a wing polymorphism (Roff, 1990), indicating that cricket genetic architecture is capable of such associations.

Kauai males show a similar pattern of reproductive tissue investment associated with the wing mutation, where *normal-wing* males possess heavier testes than *flatwing* males (Bailey et al., 2010; Rayner et al., 2019). Kauai males also exhibit an inconsistency between potential and actual reproductive outcomes, as *flatwing* males sire more offspring than *normal-wings* when mated to a *normal-wing* female (Heinen-Kay et al., 2019). At present, the mechanism underlying this discrepancy is unclear in both males and females. Other possible explanations include differences in the timing of oogenesis, egg viability or hatching success, or temporal pattern of egg laying associated with female genotype. A fruitful direction for

TABLE 2 Results of general linear models examining differences in log-transformed reproductive tissue mass of *Teleogryllus oceanicus* females from Kauai, Oahu, and Mangaia reared in either the presence or absence of calling song

Effect	F	df	p
Acoustic treatment	25.7966	1, 280	<.0001
Population	2.5686	2, 280	.0784
Acoustic treatment \times Population	3.1978	2, 280	.0424
Log soma mass	55.0464	1, 280	<.0001
Age	25.0709	1, 280	<.0001

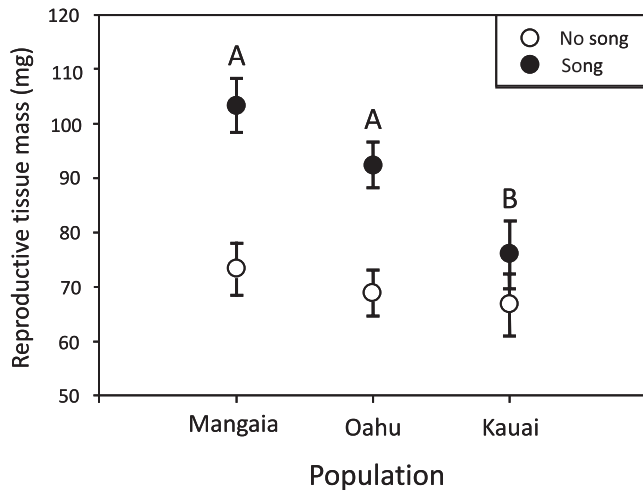


FIGURE 2 After controlling for soma mass and age, reproductive tissue mass of female *Teleogryllus oceanicus* from Mangaia and Oahu, but not Kauai, differed significantly when reared in the presence (“song”; closed circles) or absence (“no song”; open circles) of male calling song. LS means \pm SE depicted

future research would be to compare lifetime reproductive success between homozygous *flatwing*, homozygous *normal-wing*, and heterozygous females mated with *flatwing* and *normal-wing* males. This would shed light on potential interactive effects of male and female genotypes on reproductive success, and the reproductive schedules associated with each allele.

Female *T. oceanicus* from Mangaia and Oahu, but not Kauai, showed a plastic reduction in reproductive investment after being reared in a song-less social environment characteristic of a population harboring many *flatwing* males or few singers. Because this plasticity was expressed in two populations that differ in evolutionary history with the parasitoid and *flatwing* phenotype – neither the fly nor the silent male morphs exist in Mangaia, but both occur in Oahu – it suggests that it represents an ancestral response to social cues during rearing. Low investment in reproduction in song-less conditions should be adaptive in ancestral conditions where all males are capable of singing. In this scenario, lack of conspecific song indicates that males, and thus mating opportunities, are exceedingly rare and it would be prudent to invest in other areas. Because our Mangaia colony has been in the laboratory for eight years prior to data collection for the current study without supplementation from the wild, it is possible that adaptation to laboratory conditions or inbreeding may have occurred. However, because Mangaia females responded very similarly to those from Oahu, we do not believe this is likely to have impacted our results.

Kauai females showed no physiological plasticity, and generally exhibited low levels of reproductive investment similar to Mangaia and Oahu females reared without song exposure. This result is in line with another recent study that failed to find socially-induced reproductive plasticity in Kauai *T. oceanicus* females (Lierheimer & Tinghitella, 2017). Kauai is the only population where crickets have experienced nearly song-free conditions for over 15 years (~60 generations). Formerly plastic physiological processes may have become

fixed in Kauai after over a decade of selection in silence (Pfennig et al., 2010; West-Eberhard, 2003). This is consistent with the “plasticity first” model of evolution, where plasticity in trait expression exists in ancestral clades or populations, but expression of the trait becomes fixed through genetic assimilation (Levis & Pfennig, 2016). In contrast to females, Kauai males do show socially-induced physiological plasticity in reproductive tissues, where males raised with acoustic cues invest more in testes size and sperm viability (Bailey et al., 2010). It is unclear why the sexes would differ in reproductive physiological plasticity, but other studies have also found sex-specific differences following the spread of *flatwing* in Kauai. For instance, Kauai females show less socially-induced plasticity in gene expression (Pascoal et al., 2018) and walking behaviors (Balenger & Zuk, 2015; Heinen-Kay, Strub, & Zuk, 2018) than males.

Differences in both acoustic environment and the alleles underlying the silent male morphs in Kauai and Oahu could contribute to their divergent evolutionary dynamics of sexual signal loss. In Oahu, ~50% of males exhibit the *flatwing* phenotype, and calling song is commonly encountered in the field. This persistent difference in acoustic environment between Kauai and Oahu should impose different selection regimes on the crickets. In particular, lack of acoustic conspecific cues during rearing are known to reduce female choosiness (Bailey & Zuk, 2008; Swanger & Zuk, 2015). Over many generations, if Kauai females are consistently less choosy during mate choice than Oahu females, this will drastically weaken selection on both male song and female preferences, facilitating the spread of silent males in Kauai. In addition, the allele underlying the *flatwing* phenotype differs between Oahu and Kauai. While both male *flatwing* morphs are caused by mutations on the X-chromosome, they are associated with different variants along the chromosome and have independent origins (Pascoal et al., 2014). A productive direction for future research would be to investigate whether the pleiotropic effects of the two *flatwing* mutations are the same in each population. If, for instance, the Oahu *flatwing* allele is associated with a more severe reproductive cost for females than we found for Kauai, it could help explain why the proportion of *flatwing* males present in Oahu has consistently remained lower.

When an allele benefits one sex, it may be costly in the other sex (producing intralocus sexual conflict) or it could be beneficial in the other sex (favoring its spread). Our results suggest that the *flatwing* allele in Kauai and the environmental consequences of the silent male phenotype has elements of both of these outcomes. This more complex scenario might, perhaps paradoxically, help explain how *flatwing* males have persisted in wild cricket populations. On both Kauai and Oahu, *flatwing* males appear to rely on the remaining callers to support their alternative mating tactic of satellite behavior (Bailey et al., 2010; Zuk et al., 2006). If *flatwing* were to reach fixation in either population, then that population could easily go extinct since the sexes would be largely unable to locate each other. Hence, if females unambiguously benefitted from carrying the *flatwing* allele, it might spread to the point of extirpating the *normal-wing* males. Conversely, if females paid too large a cost for bearing the allele, it might not have been able to establish a foothold, and the benefit to males might not have been realized. The more nuanced effects of the

flatwing allele in Kauai females should contribute to the maintenance of the polymorphism in males. Our study demonstrates that females are not simply neutral passengers along for the ride of male sexual signal evolution, and suggests that the consequences of sexual signal evolution for the non-signaling sex in wild populations may be more complex than previously appreciated.

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DATA ARCHIVING

If accepted, our data will be deposited in Dryad Digital Repository. <https://doi.org/10.5061/dryad.v732vb1>

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