

## Research



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## Animal behaviour

# Obligately silent males sire more offspring than singers in a rapidly evolving cricket population

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How sexual traits are gained and lost in the wild remains an important question in evolutionary biology. Pacific field crickets (*Teleogryllus oceanicus*) in Hawaii provide an unprecedented opportunity to investigate the factors facilitating evolutionary loss of a sexual signal in real time. Natural selection from an acoustically orienting parasitoid fly drove rapid evolution of a novel, silent male morph. While silent (flatwing) males enjoy protection from the fly, they face difficulty attracting mates. We tested how offspring production varies in association with three male attributes affected by the spread of flatwing: wing morph (flatwing or normal-wing), age (flatwings should survive longer than singers) and exposure to calling song during rearing (wild populations with many flatwings lack ambient calling song). Per mating event, flatwings sired more offspring than singers and older males were mounted more quickly by females when presented with standard courtship song. Despite prior work showing that male age and acoustic experience influence sperm characteristics associated with fertilization, age and song exposure had no influence on male offspring production per mating. This represents the first evidence that the silent male morph possesses a reproductive advantage that may help compensate for precopulatory barriers to mate attraction.

## 1. Introduction

The evolutionary dynamics of sexual signals—emergence of new signals or loss of existing ones—are important to understand because such traits are critical for mate attraction and are often implicated in speciation [1,2]. Few examples of contemporary sexual signal evolution in wild populations exist [3], making it difficult to study how gains or losses of sexual traits become established in nature.

Pacific field crickets (*Teleogryllus oceanicus*) in Hawaii provide an unparalleled opportunity to investigate factors that influence sexual signal loss because of the recent emergence of a silent male morph. Like most crickets, *T. oceanicus* males stridulate to produce a long-range calling song and short-range courtship song that attracts females. In Hawaii, a deadly acoustically orienting fly, *Ormia ochracea*, exploits male calling song to locate its cricket host [4,5]. The fly deposits mobile larvae on and near the cricket, which burrow inside and kill it within a week [6]. Between 2001 and 2003, natural selection from the fly has driven rapid evolution of a novel wing mutation that renders males obligately silent [7]. The wing mutation arose and became established at approximately 90% of the population in Kauai (HI) in less than 20 generations [7]. These silent (flatwing) males appear to enjoy a survival benefit by avoiding the fly, but cannot sing to attract females [7]. Flatwings can achieve matings by employing an alternative mating tactic, satellite behaviour, where males intercept females responding to other males' calls [7–9].

Although females prefer not to mate with flatwings [10], these silent males have been highly successful. Past research has helped clarify how flatwings overcome precopulatory barriers to mating, but little is known regarding how they fare after mating has occurred. If flatwings possess an advantage that allows them to achieve greater reproductive success, it could help compensate for weak female attraction and at least partially explain their rapid spread in Hawaii.

We investigated how three male attributes affected by the spread of flatwing influence offspring production in *T. oceanicus*: (1) wing morph, (2) age at mating and (3) exposure to conspecific song during rearing, while controlling as many other aspects of the mating process as possible. First, inherent differences between flatwing and singing (normal-wing) morphs might influence reproductive success. Recent studies have revealed behavioural [9], physiological [8,11] and gene expression [12,13] differences associated with wing morph. Notably, flatwings exhibit more feminized gene expression profiles [13] and smaller testes than normal-wings [8], though reproductive tissue investment does not always predict fertilization ability [14]. At the same time, theory posits that flatwings should invest more in their ejaculate because they face greater difficulty attracting females than singers as they rely on satellite behaviour [15,16]. We suspect that wing morph may be associated with differential reproductive success because of pleiotropy or linkage with the mutation, or correlated evolution, but the predicted direction is unclear.

Second, because flatwings avoid the parasitoid, they should live longer than normal-wings in the wild [7]—male *T. oceanicus* from non-parasitized populations are older on average than those from parasitized islands [17]. Older *T. oceanicus* have greater sperm viability (an important predictor of fertilization [18]) and a different composition of seminal fluid proteins (which influence sperm viability, fertilization and female reproductive physiology [19,20]) than younger males [14]. Furthermore, older male field crickets tend to experience greater pairing [21,22] and competitive fertilization success [23] than younger males. We thus predict that older males will have greater reproductive success than younger males.

Finally, cricket populations with many flatwings have low levels of conspecific song. Hearing songs of rival males should lead to increased reproductive investment owing to high perceived risk of sperm competition. Male *T. oceanicus* reared with exposure to calling song develop larger testes, exhibit greater sperm viability, show increased gene expression of seminal fluid proteins and shift mating tactics compared with males reared in acoustic isolation [8,24,25]. Thus, we expect males reared with abundant song exposure to sire more offspring than those reared without social cues. If flatwings, older males, or those raised without song sire disproportionately more offspring, it could help explain how flatwings rapidly spread in Hawaii despite weak female preference.

## 2. Material and methods

### (a) Cricket rearing

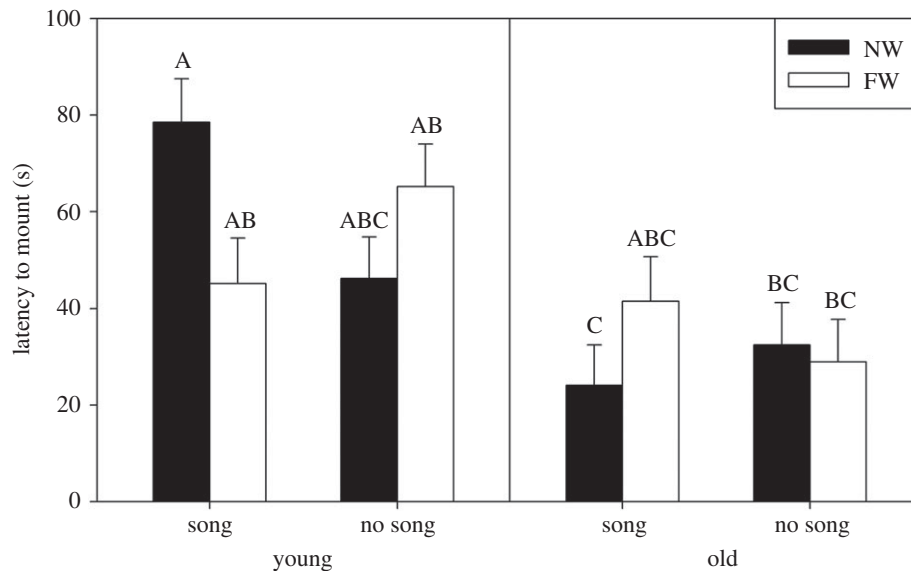
The Kauai laboratory colony was founded in 2003 after discovery of flatwing and has been supplemented at least annually with wild eggs. In 2013, we began constructing separate colonies descended from Kauai that breed true for wing morph (see

electronic supplementary methods). Crickets were reared in temperature- and humidity-controlled Caron incubators set to a 12–12 photo-reversed LD cycle. We isolated late instar juvenile males from the flatwing and normal-wing colonies at least two weeks prior to adult eclosion in 118 ml individual rearing containers with rabbit chow, water vial and egg carton for shelter. Males were randomly assigned to age ('young' or 'old') and acoustic ('song' or 'no song') treatments. 'Young' males were mated 6–10 days post-eclosion, and 'old' males were mated 16–20 days post-eclosion. These were selected based on age distributions in wild populations [17]. The 'song' acoustic rearing treatment was created by broadcasting a continuous loop of Kauai calling song at 80–85 dB sound pressure level (SLP) (measured at the cup lid with an AZ sound-level meter model 8922) from three Sony CD players connected to six speakers distributed throughout a foam-lined incubator. The 'no song' incubator was identical except that no song was played. Acoustic treatments and corresponding crickets were periodically swapped to avoid incubator effects. Crickets were checked daily for adult eclosion. Normal-wing males were surgically muted by removing the scraper from the forewing to control for presence/absence and quality of courtship song perceived by females. The same procedure was performed on flatwings to control for handling effects.

Males were mated with females from the normal-wing colony. Juvenile females were reared in a 5.7 l container and checked daily for eclosions. Adult females were transferred to individual 118 ml rearing cups and maintained in an incubator containing singing males until the mating trial 6–12 days post-eclosion.

### (b) Mating trials

Mating trials occurred in a 12 × 17 cm plastic container with 10 cm high walls in a 21–26.5°C anechoic room during the cricket's normal active period (09.00–21.00). A fresh piece of paper towel was placed on the arena floor to minimize accumulation of chemical cues. Immediately after placing crickets in the arena, we began playing a recording of Kauai courtship song broadcast at 65–70 dB (measured 10 cm from the speaker) through a mesh-covered opening in the mating arena. This allowed us to control for perceived differences in attractiveness mediated by song quality that may affect reproductive outcomes. We measured female latency to mount, a common metric of female preference [26,27], as the time from courtship song onset until the female mounted the male. The trial was terminated if mounting failed to occur within 5 min. Each cricket was allowed up to three mating opportunities at least 1 day apart, paired with a different individual, before being discarded from the experiment. If a spermatophore was transferred, we guided the female into a 118 ml container, then used forceps to remove the spermatophore after 30 min. Sperm transfers linearly with time [28], and spermatophore removal allows females to exert postcopulatory mate choice [29]. To control the amount of sperm received by each female, we excluded females that prematurely removed the spermatophore. Following spermatophore retention and removal, the female was transferred to an 11 cm<sup>2</sup> plastic container with 6 cm high walls containing food, egg carton and moist cotton for oviposition, housed in the 'no song' incubator. After 7 days, we removed the female and measured pronotum width to the nearest 0.01 mm using digital calipers. Eggs in moist cotton remained in the incubator to hatch. Hatchlings were counted at least five times per week, and cotton was discarded after no hatchlings emerged for one week. Of 241 females that mated and had opportunity to oviposit (29–31 females per treatment combination; see electronic supplementary material, table S1), 215 yielded offspring.



**Figure 1.** Overall, female *T. oceanicus* were faster to mount older males than younger ones when presented with a standard courtship song. ‘Song’ and ‘no song’ refer to acoustic rearing treatments. Filled bars represent flatwings (FW); open bars represent normal-wings (NW). Least squares means  $\pm$  s.e. from untransformed data are depicted. Letters denote Tukey HSD levels from ANCOVA on cube-root transformed latency to mount.

### (c) Data analysis

To test whether any of our main effects (male wing morph, age category and acoustic experience) were associated with whether the female mounted the male, we performed a generalized linear mixed model with binomial error distribution in R v3.4.4 (R Core Development Team, <http://www.R-project.org/>). Male and female IDs were included as random effects because some crickets had multiple mating opportunities. We used logistic regression to test whether mating failure, where a mating yields no offspring, was associated with the covariates (female and male pronotum width, and female age) or main effects listed above. Among matings that produced offspring, we used an analysis of variance (ANCOVA) with Type I sums of squares (i.e. sequential tests) to test whether female latency to mount (cube-root transformed to improve normality) differed in association with the covariates and main effects described above. Tukey HSD was employed to disentangle group differences for significant interactions among main effects. To test for differences in offspring production, we again used a Type I ANCOVA with the same covariates and main effects. For female latency to mount and number of male offspring produced per successful mating, we also employed a forward model selection approach based on corrected Akaike information criterion (AICc), which qualitatively yielded the same results as our ANCOVAs (see electronic supplementary material, tables S2 and S3). Thus, we only present ANCOVA results here. Data met assumptions of the statistical models, which were performed in JMP Pro v14 unless otherwise noted.

## 3. Results

Neither mounting success nor mating failure was significantly associated with male wing morph, age category or acoustic experience (electronic supplementary material, tables S4 and S5). For female latency to mount, there was a significant three-way interaction among the main effects, which appears largely driven by male age (table 1 and figure 1). Females mounted old males approximately 2 $\times$  faster than young ones (figure 1). Per mating event, flatwing males sired significantly more offspring than normal-wings (table 1 and figure 2), though neither male age nor acoustic treatment affected reproductive success (table 1). Older females and larger crickets of both sexes produced more offspring (table 1).

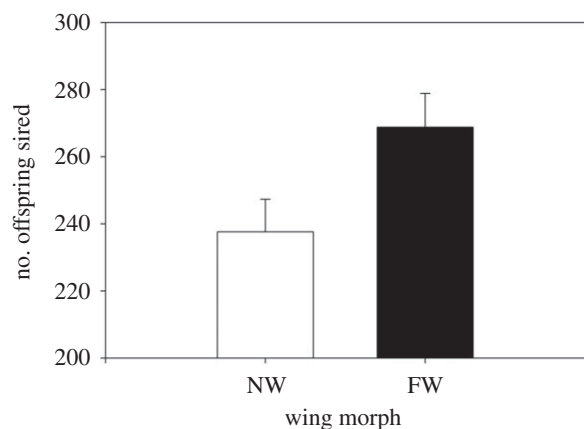
**Table 1.** Results of separate Type I ANCOVAs examining variation in female *T. oceanicus* latency to mount and number of offspring sired per successful mating. *p*-values < 0.05 are italicized.

source	latency to mount		no. offspring sired	
	$F_{1,203}$	<i>p</i>	$F_{1,203}$	<i>p</i>
female pronotum width	0.885	0.348	19.002	<0.001
male pronotum width	0.027	0.870	8.392	0.004
female age	0.013	0.910	8.228	0.005
acoustic treatment (song)	0.806	0.370	0.053	0.819
age category (age)	24.851	<0.001	0.005	0.946
wing morph	0.470	0.494	4.846	0.029
song * age	2.489	0.116	1.935	0.166
song * wingmorph	<0.001	0.991	0.053	0.818
wingmorph * age	1.801	0.181	0.065	0.799
song * age * wingmorph	9.292	0.003	0.023	0.880

## 4. Discussion

The goal of our study was to understand how three male attributes affected by sexual signal loss influence reproductive success in a rapidly evolving cricket population. Per successful mating, flatwings sired more offspring than males capable of singing. Wing morph, male age and acoustic experience interacted to exert a complex influence on female latency to mount. The clearest pattern from this interaction is that females mated more readily with older males, which should be mostly flatwings in the wild [7] and is common in crickets [21,22]. Our study provides the first evidence that *T. oceanicus* flatwings experience a reproductive advantage that should help compensate for precopulatory mating barriers.

It is unclear whether male morphs inherently differ in reproductive capacity, females bias fertilization toward



**Figure 2.** Flatwing (FW) males sired significantly more offspring than normal-wing (NW) males descended from Kauai. Least squares means  $\pm$  s.e. depicted.

flatwings or genomic interactions between the sexes influence reproductive success. It is possible that the morphs differ in sperm viability owing to pleiotropy or linkage with the locus responsible for obligate silence, or exhibit differential gene expression of seminal fluid proteins. Another possibility is that flatwings are favoured during cryptic female choice—female field crickets are known to bias paternity in favour of unrelated [30,31] and attractive [32] males. Though we controlled for differences in attractiveness mediated by courtship song, the morphs express different cuticular hydrocarbons [11], olfactory signals used in sexual communication, though it is unclear whether they differ in attractiveness [33]. Prior work has shown that male quality has a greater impact on offspring viability than genetic interactions between *T. oceanicus* parents [34], and homozygous flatwing and normal-wing females do not produce different numbers of offspring when mated with a flatwing male [35]. We thus

believe that an outcrossing advantage is unlikely to explain our results.

Despite evidence that socially mediated cues of sperm competition risk and male age influence testes size, sperm viability and seminal fluid protein composition [8,14,24,25], neither of these factors affected male offspring production. This suggests that the relationship between male reproductive physiology and fertilization success is not straightforward. Other studies have found conflicting results; for instance, Gray & Simmons [24] found that male *T. oceanicus* reared with song exposure had more viable sperm than crickets reared in silence, but did not produce more offspring.

Flatwing males cannot sing and experience discrimination during mate choice [9], yet have been highly successful in Kauai. Our study shows that flatwings possess a reproductive advantage that can help them overcome pre-copulatory challenges and may partially help explain how sexual signal loss rapidly evolved in Hawaiian *T. oceanicus* populations.

**Data accessibility.** Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.f28s554> [36].

**Authors' contributions.** J.L.H.-K. and M.Z. designed the study. J.L.H.-K. and E.M.U. collected the data. J.L.H.-K. analysed the data and drafted the manuscript. All authors provided critical input on the manuscript, approved it for publication and agree to be accountable for its contents.

**Competing interests.** We declare we have no competing interests.

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