LETTER

Behavioural plasticity compensates for adaptive loss of cricket song

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Abstract
Behavioural flexibility might help animals cope with costs of genetic variants under selection, promoting genetic adaptation. However, it has proven challenging to experimentally link behavioural flexibility to the predicted compensation of population-level fitness. We tested this prediction using the field cricket *Teleogryllus oceanicus*. In Hawaiian populations, a mutation silences males and protects against eavesdropping parasitoids. To examine how the loss of this critical acoustic communication signal impacts offspring production and mate location, we developed a high-resolution, individual-based tracking system for low-light, naturalistic conditions. Offspring production did not differ significantly in replicate silent versus singing populations, and fitness compensation in silent conditions was associated with significantly increased locomotion in both sexes. Our results provide evidence that flexible behaviour can promote genetic adaptation via compensation in reproductive output and suggest that rapid evolution of animal communication systems may be less constrained than previously appreciated.

KEYWORDS
adaptation, behavioural plasticity, evolutionary rescue, movement ecology, phenotypic accommodation, trait loss

INTRODUCTION

The role of behaviour in adaptive evolution remains controversial despite over a century of research (Bailey et al., 2018; Baldwin, 1896; Duckworth, 2009; Wcislo, 1989; West-Eberhard, 1983, 2005). Behaviour's 'flexibility' is at the centre of this debate. It has been argued that behaviour's context dependence and variability within genotypes enable individual organisms to cope with changing environmental conditions, reducing the efficacy of selection and slowing genetic adaptation at the population level (Losos et al., 2006). In contrast, behavioural flexibility could expose new beneficial phenotypes to the action of selection, eventually leading to their fixation and promoting evolutionary adaptation (Levis & Pfennig, 2016; West-Eberhard, 2005). The third potential influence of behaviour combines these processes: adaptive mutations can have indirect costs arising from negative, or antagonistic, pleiotropy (Fisher, 1958; Orr, 1998, 2005; Williams, 1957), and flexible behaviours could mitigate these for long enough to allow such mutations to spread

under selection (Bailey et al., 2021; West-Eberhard, 2005; Zuk et al., 2014). This process has arguably received the least attention, and it usually remains unknown whether, or how, flexibility in behaviour mitigates negative fitness effects of new adaptive variants.

To address this, it is necessary to overcome challenges associated with identifying relevant behaviours during an episode of adaptive evolution, measuring them under ecologically realistic conditions, and experimentally linking them with population-level fitness outcomes. For example, differences between field and laboratory environments can alter inferences about animal behaviour in unexpected ways. Consider mating behaviour: the physical environment can impair or facilitate sexual signalling, climatic variation can favour different mating strategies, and seasonal dynamics can alter mate preferences (Candolin, 2019; Chaine & Lyon, 2008). The challenge is to increase the reliability of inferences about behaviour's role during adaptive evolution by combining the control, replication, and resolution of experimental laboratory manipulations with the biologically realistic context of the environment that

shaped the behaviour's evolution in the first place (Archard & Braithwaite, 2010; Calisi & Bentley, 2009).

We used such a combined approach to study how adaptive changes in communication behaviour can be facilitated in an insect system that is an exemplar of rapid evolution in the wild. In Hawaii, the field cricket *Teleogryllus oceanicus* co-exists with a lethal parasitoid fly (*Ormia ochracea*). Female flies locate male crickets by detecting and orienting towards their song (Müller & Robert, 2001) and then release larvae that burrow into and consume the host (Zuk et al., 1993). Genetic variants that silence male crickets have rapidly spread through Hawaiian populations over the last 20 years (Pascoal et al., 2014; Rayner, Aldridge, et al., 2019; Zhang et al., 2021; Zuk et al., 2006, 2018). One of these, *flatwing*, is an X-linked trait that segregates as a single locus and causes males (which are hemizygous for the X chromosome) to develop severely altered forewing structures. As crickets sing with their forewings, flatwing males are silenced and thus protected from the eavesdropping parasitoid (Zuk et al., 2006).

The genotype causing flatwing morphology is associated with fitness costs arising from associated impacts on other traits. Silent males are unable to produce calling songs to attract mates from a distance, have reduced courtship and mating success (Bailey et al., 2008; Heinen-Kay, Strub, et al., 2019; Tanner et al., 2019), fare worse in agonistic encounters with other males (Logue et al., 2010), and develop smaller testes (Bailey et al., 2010; Rayner, Pascoal, & Bailey, 2019; Richardson et al., 2021). They also continue to express energetically costly wing movements associated with sound production, even though these no longer produce audible signals (Rayner et al., 2020; Schneider et al., 2018). At least some of these costs are mitigated by behavioural responses to the altered social environment caused by the evolutionary spread of silent males. For example, females that develop without perceiving song in their environment respond more quickly to male song playbacks, compared to females exposed to male song during development (Bailey & Zuk, 2008). In some populations, including the one studied here, males reared in silence move more (Balenger & Zuk, 2015) and are more likely to adopt satellite mating tactics by intercepting females responding to any remaining singing males (Bailey et al., 2010). Experience of silent conditions also causes both sexes to increase flight attempts (Sturiale & Bailey, 2021). These flexible responses may enhance mating success in predominately flatwing populations by facilitating female access to the remaining singing males or to satellite males near them.

Given the role of silent environments in modulating *T. oceanicus* behaviours relevant to mating success, we aimed to definitively test whether such behavioural flexibility has a compensatory role that facilitates genetic adaptation. This required two innovations. First, it was necessary to determine whether crickets experiencing silent environments for realistic lengths of time maintain fitness. That is, holding all other factors constant in a setting that approximates natural conditions—including demographics, environmental variables, and a suitable amount of time—do populations comprising only silent flatwing males achieve similar reproductive output as populations containing only normal-wing, singing males? Few studies of behaviour's role in adaptive evolution have demonstrated this important pattern of fitness compensation. Second, we evaluated flexible behaviours potentially underlying such compensation. Given the role of locomotion in almost all the studies mentioned above, we expected that behavioural compensation for the lack of acoustic signalling might take the form of increased movement.

We developed a system to individually track crickets using still image photography under conditions approximating those experienced by crickets in nature, including low light. We then tracked test populations that we manipulated to contain only normal-wing, singing males (hereafter 'Singing' treatment) or flatwing, silent males (hereafter 'Silent' treatment). If behavioural flexibility does not mitigate the negative fitness effects of *flatwing*, crickets in the Silent treatment were predicted to achieve lower reproductive output compared to those in the Singing treatment. In that case, we expected that crickets in both treatments would move similar distances and show similar social encounter rates. Alternatively, if behavioural flexibility does mitigate negative indirect consequences of *flatwing*, then we predicted similar offspring production in both the Silent and Singing treatments and an associated difference in movement behaviour.

MATERIALS AND METHODS

Laboratory tracking system

An array of nine cameras (Nikon D3300 with Sigma 17–50 mm f/2.8 lenses) was secured to the ceiling 2 m above the floor of a sealed room (Figure 1a,b). Cameras took synchronized photographs once every 2 s using radio triggers (Pixel TW-283/DC2). Images were stored on SD cards and manually transferred after trials via USB cable. Figure S1 shows a schematic plan of the tracking system.

The tracking arena measured 4.2 × 3.0 m and was kept at a constant temperature of 23°C. The arena was confined by four 60-cm-high black plastic barriers, and grass turf (Turffit Ltd., standard lawn) was laid on the floor and kept short (<5 cm) and damp. Three 30 × 30 cm foam soundproofing sheets were attached to each wall to reduce acoustic reflections. To minimize edge effects caused by wall-following behaviour, drainpipes were quartered lengthwise and fixed between the turf and the plastic walls, creating curved, rather than 90°, profiles (Figure S2). A microphone (Sony PCM-M10) recorded audio to determine when male crickets in the normal-wing treatment were singing.
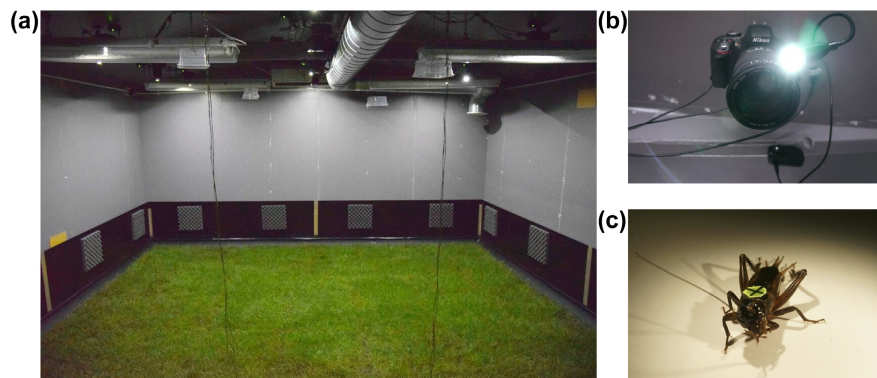
FIGURE 1 (a) Cricket tracking arena. (b) Ceiling-mounted camera with radio trigger and USB light. (c) Cricket tagged with marked retro-reflective tape.

Crickets were individually identified using 3×3 mm retro-reflective tags (Oralite Reflexite tape) marked with hand-written symbols. Tags were less than 1 mm thin and less than 0.01 g (<2% of the mass of a typical cricket). We attached them to crickets' pronota using gel superglue (Loctite) (Figure 1c). The retro-reflective tagging material does not scatter light laterally, so crickets in the arena could not perceive the reflected light from their own or other crickets' tags. USB LED lights were fitted next to each camera (Figure 1b). Informed by field measurements (see below, and Table S1), we positioned lights to emit 6 LUX on the arena floor so that only the reflected tags were visible in photographs. A full moon can reach ca. 0.2 LUX, while sunset/sunrise is typically ca. 400 LUX (Kyba et al., 2017). Six LUX is therefore comparable to post-sunset/pre-sunrise conditions when crickets are most active, and to artificial light from streetlamps common in their environment. Acoustic signals are the only known signal modality for long-range mate location in *T. oceanicus*, and the grass substrate would have prevented visual detection over non-trivial distances. Image processing and tracking algorithms were custom coded in MATLAB version 2016b (MathWorks Inc., 2016). Tags were automatically extracted from the black background in each raw image and identified, and then tracks of individual crickets were assembled. Code is available at github.com/wtschneider/cricket-tracking.

Experimental design and parameterization

We used the tracking system to measure reproductive output and a suite of movement-related behaviours in replicate test populations experimentally manipulated to contain all normal-wing, singing males (Singing treatment) or all-flatwing, silent males (Silent treatment). To approximate conditions experienced by crickets in nature, we collected environmental and demographic reference data from five field sites across three Hawaiian islands in 2016 (Figure S3). After sunset, we surveyed cricket densities within 10 haphazardly placed 5.6×4.2 m grids at each site, from which we could later calculate rescaled densities for our 4.2×3.0 m laboratory tracking arena (see above). Our protocol ensured that every cricket within a grid was counted, and counted only once, by pre-defining a primary search period of 15 person-search-minutes (the minimum effort required for a plot) and a secondary search period of 9 person-search-minutes required to elapse *without* finding a cricket. If a searcher located a cricket within the secondary search period, the 9 person-search-minute clock was reset and the procedure repeated until the minimum secondary search time was reached. Cartesian coordinates and demographic attributes of each cricket (adult female, adult normal-wing male, adult flatwing male, nymph) were recorded. Temperature and light levels were recorded at ground level using a digital thermometer and a light level meter (CEM DT-1300).

Cricket origins and rearing

The laboratory population of crickets was originally derived from a population in Kauai that contained singing males in the 1990s and, following the initial detection of the *flatwing* mutation in 2003, now contains only silent flatwing males (Rayner, Aldridge, et al., 2019; Tinghitella et al., 2018). In field surveys, we found no normal-wing males in any Kauai survey plot (see Results) consistent with a scenario in which the *flatwing* allele had fixed in approximately 2016 (Rayner, Aldridge, et al., 2019; Tinghitella et al., 2018). Thus, our laboratory experiment assessed the two demographic situations representing evolutionary optima before and after the emergence and spread of flatwing crickets.

Crickets were reared in mixed-sex 16-L plastic containers (approximately 30 per box) in common-garden conditions at 25°C on a 12h:12h photo-reversed light:dark cycle following an established protocol (Bailey & Macleod, 2014). Twice a week, boxes were cleaned, and food (Burgess Excel Junior and Dwarf rabbit pellets), water (moist cotton) and cardboard egg carton shelter

were replenished. Virginity was ensured by removing individuals to single-sex stock boxes of standard density upon adulthood. Flatwing and normal-wing males were kept together to control their previous experience. As age can affect behavioural and fitness traits, we minimized confounding variation by ensuring that all subjects were tested between 21 and 29 days post-eclosion. In closely related *Gryllus pennsylvanicus*, age distributions in nature are centred around 15 days and range upwards to 30 days late in the season (Zuk, 1987); similar demographics are reasonable to expect in the continuously breeding species we studied.

Experimental subjects were sourced from crosses involving three replicate pure-breeding *normal-wing* and three replicate pure-breeding *flatwing* laboratory lines that were originally derived from the population on Kauai, as described elsewhere (Pascoal et al., 2016). As *T. oceanicus* has XX/XO (female/male) sex determination and *flatwing* is X-linked and male-limited, male genotype can always be detected visually, whereas female genotype cannot. To produce an admixed population for our experiment, we performed two generations of crossing by reciprocally mating all combinations of lines. From this, we haphazardly selected test subjects. Male morphotype was determined through visual inspection, while female genotype was unknown and therefore mixed, such that the genetic background of all crickets was randomized except for whether males carried the silencing *flatwing* mutation or not. The parasitoid fly was not included in this experiment.

Tracking experiment

Tracking experiments were conducted between 20 March and 5 April 2017. We tracked eight crickets per trial. Cricket density in the arena (0.64 crickets per m^2) approximated the density of a Hawaiian cricket population from the Big Island (Figure S1). This population was used as a reference because of its high proportion of singing males, reflecting a situation without disruption caused by acoustic signal loss. Although crickets were constrained within the arena during trials, in nature we expect that similar rates of movement into and out of a bounded area the size of the arena would maintain a constant density of crickets, though encounters with new individuals could potentially occur. While crickets in our experiment might have experienced a higher probability of repeatedly encountering the same individual, this would have affected both treatments equally. Factors such as prior social experience, acclimation period, genetics, habituation, and natural variation in responsiveness were controlled in our experiment, potentially limiting the generalizability of our findings at higher or lower rearing densities (Webster & Rutz, 2020). Our approach minimized the potential for confounds to examine the experimental treatment (Singing vs. Silent) under conditions approximating those found in nature. Stock crickets were originally derived from eggs laid by females haphazardly sampled in the field, representing both the genotypes of those females and any males they had mated with.

Eight crickets were tracked per experimental trial: four females and four males. In the Silent treatment, the four males were silent flatwing morphs, and in the Singing treatment, the four males were normal-wing morphs capable of singing. The evening before each trial, the eight test subjects were haphazardly selected from stock boxes, weighed, tagged, and isolated into 100-mL tubs with cardboard shelter, food, and water. Although there is a possibility that reaching into stock boxes to haphazardly obtain test subjects could have inadvertently selected bolder or less mobile individuals, any such sampling bias would have affected both treatments equally. Females and males had different tag colours, with four uniquely identifying symbols randomly assigned within each sex. Experiments began at 08:00 AM each morning, which aligned with time-of-sunset under the photo-reversed light:dark cycle.

One trial was performed per day, and Silent and Singing treatments were alternated. Crickets were placed into the arena in low light under one of four release boxes positioned approximately 1 m from each corner controlled by a pulley system fixed to the ceiling (Figure S2). Release boxes were located to avoid interference with the field of view of the cameras and other hardware fixed to the ceiling. Two crickets of the same sex were placed under each box. The crickets were left to habituate for 5 min before camera triggering started. After a further 5 min, the boxes were simultaneously raised. This meant that, briefly at the beginning of a trial, same-sex pairs were closer to one another than to any other cricket, but starting conditions were identical across all trials and treatments to avoid confounding effects. Each trial ran for 3 h. A total of 16 experimental trials were run, evenly split across treatments. If a tag was lost or a camera malfunctioned during a trial, the trial was omitted. This resulted in three trials being removed, leaving seven Silent and six Singing trials for analysis.

Immediately after trials, each female was isolated in a 1-L container with food, shelter, and moist cotton pads for oviposition. Females were given 2 weeks to oviposit and then removed, and eggs were given three further weeks to hatch. Hatchlings were then removed, frozen, and counted.

Data analysis

We calculated *distance moved* and *time spent stationary* for every cricket. To assess whether movement was associated with the presence of male song in the Singing trials, we detected periods of singing in audio recordings using an automated frequency power threshold. To

identify these, the power spectral density of frequencies centred at ca. 4.8 kHz, the dominant frequency of *T. oceanicus* (Bennet-Clark, 2003), was extracted using a Fast Fourier Transform. To investigate the potential for social interactions (such as mating, aggression, or evaluation) to occur, we used a measure of how much time any individual cricket spent within a 10-cm radius of another ('encounters'). Our setup did not allow us to detect known physical contact or determine what sort of social behaviour occurred.

Our statistical approach was designed to test whether there were treatment differences in offspring production, movement distance, and the positioning of crickets near enough to one another for potential social interactions. Offspring production was analysed in two ways. The first analysis assessed variation in female offspring number between treatments, and the second assessed variation in whether individual females produced any offspring or not. Offspring numbers varied considerably (ca. 55% of females did not produce any offspring), so for the first analysis we used a zero-inflated generalized linear model (GLM) with a Poisson distribution using the glmmTMB package (version 1.1.3) in R. *Treatment* was a fixed factor, *trial* a random effect, and cricket *mass* and *age* covariates. The second analysis used a zero-inflated negative binomial model with the same predictors as above.

Movement data were analysed using linear mixed effect models (LMEs). Data were normalized using transformations where necessary (square root for *time spent near other crickets*, and log for *distance moved*). Social encounter data were analysed using generalized linear mixed models (GLMMs) with gamma distributions. *Treatment* and *sex* were fixed factors, *trial* a random effect, and *mass* and *age* covariates. A *treatment * sex* interaction term was included if it improved model fit (i.e. reduced AIC). Plots were created using notBoxPlot from MathWorks File Exchange (Campbell Rob, 2022).

RESULTS

We found no significant difference in population-level offspring production between Silent (mean offspring $= 48 \pm 72$ s.d.) and Singing (mean offspring $= 35 \pm 51$ s.d.) treatments (zero-inflated GLM: $Z(52) = -1.3$, $p = 0.201$). Similarly, there was no significant treatment difference when considering whether females did or did not produce offspring (GLM: $Z(52) = 0.7$, $p = 0.454$; Silent treatment: 22/56 females produced offspring; Singing treatment: 24/48 females produced offspring). Regardless of treatment, heavier females produced fewer offspring (zero-inflated GLM: $Z(52) = -7.5$, $p < 0.001$), which could be related to accumulation of low-quality, unfertilized eggs. It was not possible to determine whether variance in male reproductive success differed between treatments.

Crickets in the Silent treatment moved further than those in the Singing treatment (LME: $t(11) = -2.4$, $p = 0.037$; Figure 2a; Figure S4), but there was no significant difference in time spent stationary (LME: $t(11) = 1.0$, $p = 0.362$). On average across both treatments, crickets spent 51% of their 3-h trials in active movement, and distance moved by a single cricket ranged from 21 to 340 m. Average distance moved per unit time decreased as trials progressed, from an average of 16.5 m during the first 20 min to an average of 9 m in the final 20 min (Figure S4). This pattern is consistent with the progressive acquisition of mating opportunities and resulting pause in movement and mate-searching. However, the fact that crickets in both treatments never fully ceased moving illustrates that experimental populations did not become saturated with mating encounters. These observations are important for interpreting the differences in movement as compensation for a lack of advertisement calls in the Silent treatment (see Discussion). Overall, males moved further than females (LME: $t(88) = 4.1$, $p = 0.001$; Figure 2a) and spent less time stationary (LME: $t(87) = -2.8$, $p = 0.006$). Age and body mass were not associated with movement
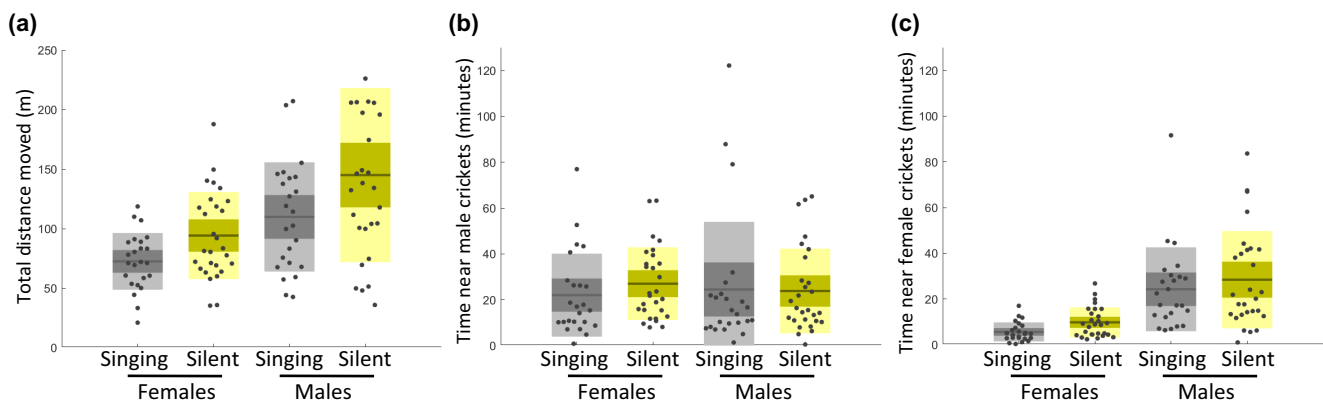

(a) Total distance moved (m) — Singing / Silent (Females), Singing / Silent (Males)

(b) Time near male crickets (minutes) — Singing / Silent (Females), Singing / Silent (Males)

(c) Time near female crickets (minutes) — Singing / Silent (Females), Singing / Silent (Males)

FIGURE 2 (a) Total distance moved during 3-h trials, with variation between experimental treatments ('Singing' vs. 'Silent') illustrated for each sex. (b) Time spent near male crickets, by sex and treatment. (c) Time spent near female crickets, by sex and treatment. Individual dots show raw data for each cricket. Central bars indicate means, the dark-shaded area indicates the standard error (95%), and the light-shaded area indicates the standard deviation.

metrics (Tables S1 and S2). An example of cricket tracking data is available in Video S1.

Crickets spent longer in close proximity to one another in the Singing treatment compared with the Silent treatment, but only when both were female (GLM: $Z(104) = -2.90$, $p = 0.004$; Figure 2c). Regardless of treatment, males spent more time near female crickets than females did (GLM: $Z(104)$: -5.12, $p < 0.001$; Figure 2c), but there was no increase in the time silent males spent near females (GLM: $Z(52)$: 0.395, $p = 0.693$). In the Singing treatment, male song was detected for an average total of 7.6 min per trial, with an average bout length of 108 s. Crickets were not more or less likely to be in active movement when song was present in the arena (LME: $t(50) = -0.75$, $p = 0.463$).

DISCUSSION

A fundamental prediction of evolutionary theory is that new mutations under selection are prone to indirect, disruptive effects arising from negative pleiotropy (Fisher, 1958; Orr, 1998, 2005). Behaviour has been thought to play an important role in buffering such effects by enhancing the adaptive potential of populations in which such mutations invade (Bailey et al., 2021; Duckworth, 2009; Pfennig et al., 2010; West-Eberhard, 2005; Zuk et al., 2014). In Hawaiian *T. oceanicus*, we found evidence that populations can maintain population-level fitness despite the loss of their main sexual signal. High-resolution individual tracking under controlled, naturalistic conditions revealed that artificial populations mimicking silent all-flatwing populations in the wild exhibited increased movement behaviour. Behavioural flexibility's contribution to the *complete* replacement of singing male crickets by an adaptive, genetically silenced form within only a few dozen generations in nature has important implications for understanding abrupt evolutionary transitions in animal communication systems.

The first requirement for such a transition to occur is that population fitness does not crash. In our experiment, population-level reproductive output was more than compensated for in the Silent treatment compared to the Singing treatment. Such restoration of fitness through the mitigation of negative indirect effects of mutation is a fundamental prediction of theories about behaviour's role in adaptive evolution (Bailey et al., 2008, 2018; Duckworth, 2009; Pfennig et al., 2010; West-Eberhard, 2005), and acoustic signalling is thought to be indispensable in species where it is the only signal modality used for long-range mate attraction. Why should population fitness not be impacted by the loss of a sexual signal that is considered vital?

It is well established that crickets of both sexes move towards callers—females for mate location and males to use satellite mating tactics (Cade, 1981; Hoy et al., 1982;

Pollack, 1982; Pollack & Hoy, 1981). It is therefore likely that increased locomotion directly contributed to the maintenance of fitness in our experiment by facilitating mate location, although we cannot definitively rule out latent factors that might be associated with movement such as changes in gene expression affecting reproductive performance. Plasticity of female mating behaviour is particularly well studied in field crickets: their mating decisions can be altered by acoustic and olfactory cues (Bailey, 2011), remembered information (Bailey & Zuk, 2009), mating experience (Rebar et al., 2011), and predation risk (Hedrick & Dill, 1993). Despite the obvious ability of females to exhibit such plasticity, the potential for satellite behaviour in males has been thought to be the key factor facilitating song loss in Hawaiian *T. oceanicus* (Bailey et al., 2008, 2010; Tinghitella et al., 2009; Zuk et al., 2006, 2018). In almost all previous studies of Hawaiian *T. oceanicus*, the benefit of behavioural plasticity to acoustic experience is presumed to derive from changes in responsiveness to male song perceived at a later time. Such benefits depend on the presence of at least one singing normal-wing male in populations where flatwing males have spread. However, song is never present in completely silent, all-flatwing populations in the wild (Rayner, Aldridge, et al., 2019; Tinghitella et al., 2018) or in the Silent treatment we designed to reproduce this condition in the laboratory. Thus, the most parsimonious explanation for fitness compensation in our study is that it is driven by locomotion differences as opposed to female mate discrimination differences. Furthermore, as the distance moved was higher in the Silent treatment, but the amount of time spent moving was roughly the same between Silent and Singing treatments, we can infer that the speed of movement was greater in the Silent treatment and potentially represented more active searching behaviour.

Despite equivalent opportunities for social encounters, females in the Silent treatment spent more time near other females than those in the Singing treatment. This was not driven by any compensatory differences in time spent with males. It could be that when there is no acoustic target for female phonotaxis, females remain near or follow other females as an alternative to undirected searching (Cade & Cade, 1992). Alternatively, the many feminized components of the flatwing phenotype, such as morphology and chemical pheromone profiles (Pascoal et al., 2020), may make it more difficult for females to distinguish other females from flatwing males. Song was detected for relatively little time (on average less than 5%) in the normal-wing Singing treatment. This low singing effort is unexpected given the assumed importance of acoustic signalling in field crickets, yet we note that it closely corresponds to a singing effort of 6% measured in an independent study of the same species (Rayner et al., 2020). The surprising infrequency of male calling raises the possibility that there is no critical dependence on song as a 'beacon' for females to locate males in *T. oceanicus*. Our experiment differed from most earlier studies of cricket calling effort in that mating was not inhibited, and males

were likely to have ceased singing once contact was made with a prospective mating partner. Additionally, the substrate through which crickets must navigate both in the wild and in our tracking arena—short, clipped lawn—may differ from ancestral conditions undisturbed by humans (being more amenable to movement-based, rather than acoustically based, mate location). This may partially explain why silent male *T. oceanicus* have so rapidly proliferated in Hawaii.

While our results provide confirmatory evidence for behaviour's facilitating role in rapid genetic adaptation, it is difficult to discern whether flexible behaviour is a uniform characteristic of all crickets in the population we tested, or whether it is genetically associated with *flatwing* alleles. All our experimental crickets were reared in a common-garden environment and only differed in the composition of male morphotypes. There is evidence that juveniles carrying *flatwing* alleles move more, and that genotypes from the Kauai population studied here show stronger plasticity of locomotion depending on acoustic experience (Balenger & Zuk, 2015; Sturiale & Bailey, 2021). Critically, female genetic background was randomized across all trials, so the differences in female behaviour we observed can be attributed to plastic responses to the presence or absence of song, or plastic responses to other indirect effects of the *flatwing* versus *normal-wing* genotype of males in the experiment. Differences in male behaviour may be due to the presence or lack of song or to their own genotype. For example, there is evidence that flatwing males sire more offspring per successful insemination, though this effect may be partly offset by differences in the rate of successful mating and cryptic female choice (Heinen-Kay, Urquhart, & Zuk, 2019). Intriguingly, such a flatwing male advantage would be consistent with the greater (though nonsignificant) offspring production in our Silent treatment containing only flatwing males.

It is reasonable to assume that energetic costs are associated with moving more. Although reproductive output did not differ significantly between treatments, the increased locomotion we observed may be associated with longer-term penalties affecting lifetime reproductive fitness. Costs of plasticity, and of plastic phenotypes, have been extensively debated but are generally considered to be minor compared with other factors that can inhibit the evolution of plasticity, such as environmental unpredictability (Auld et al., 2010; DeWitt et al., 1998; Murren et al., 2015). For the *T. oceanicus* system studied here, we consider that any costs of the mechanisms enabling plasticity, or of increased movement itself, must be offset by the benefits gained in mate location and reproduction: were such fitness costs prohibitive, selection would select against plastic genotypes in all-flatwing populations.

In Hawaiian *T. oceanicus*, acoustic signalling may be a useful but non-essential component of mate location; to our knowledge it has not been considered that such a 'soft' requirement in its own right might have facilitated the rapid spread of flatwing crickets. It would be difficult to determine whether a relaxed acceptance threshold acts as a driver or consequence of the spread of flatwing crickets; viewed in the context of prior findings, an increasingly plausible scenario is that behavioural flexibility, genetically hitchhiking locomotion differences associated with *flatwing* alleles, and relaxed acceptance thresholds all contribute to exceptionally rapid, adaptive song loss. Multiple all-silent populations of *T. oceanicus* have now been discovered, and it is noteworthy that acoustic signalling has been secondarily lost in multiple katydid and field cricket species (Bailey et al., 2019; Gray et al., 2018; Otte & Alexander, 1983). Examples of evolutionary signal loss are widespread across more distant animal taxa (Wiens, 2001), and our results raise the intriguing possibility that the flexibility of behaviours typically associated with animal signalling, in both signallers and receivers, could paradoxically enhance the likelihood of evolutionary signal loss. Where signals are under selection, rapid genetic adaptation may also be more probable via signal loss as compared to signal gain: the genetic complexity and integration required to produce an effective signal makes signal loss a large mutational target with significant potential fitness consequences. To fully understand behavioural mitigation of such consequences, it is important that the environmental and demographic context in which it has been lost is taken into account in experimental designs (Weigel et al., 2015). The ongoing development of high-resolution tracking technologies (Chen et al., 2021; Walter et al., 2021) promises to reveal further unexpected insights into behaviour's contributions to rapid signal evolution and genetic adaptation.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ele.14404.

DATA AVAILABILITY STATEMENT

Data for both the tracking (raw and processed) and Hawaii demographics are published on DRYAD [DOI: 10.5061/dryad.vdncjsz33], along with source code to produce the tracking metrics.

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