NATURAL HISTORY NOTE

Purring Crickets: The Evolution of a Novel Sexual Signal

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ABSTRACT: Opportunities to observe contemporary signal change are incredibly rare but critical for understanding how diversity is created and maintained. We discovered a population of the Pacific field cricket (Teleogryllus oceanicus) with a newly evolved song (purring), different from any known cricket. Male crickets use song to attract females from afar and to court females once near. Teleogryllus oceanicus is well known for sexual signal evolution, as exemplified by a recent signal loss. In this study, we characterized the new purring sound and investigated the role of the purr in long-distance and short-distance communication. The purring sound differed from typical ancestral calls in peak frequency, amplitude, and bandwidth. Further, the long-distance purring song facilitated mate location, though the role of courtship purring song is less clear. Our discovery of purring male crickets is an unprecedented opportunity to watch the emergence of a newly evolved sexual signal unfold in real time and has potential to illuminate the mechanisms by which evolutionary novelties arise and coevolve between the sexes.

Keywords: sexual signal, rapid evolution, evolutionary novelty, *Teleogryllus oceanicus*.

Introduction

Sexual signals are frequently the only or the most divergent traits between closely related species, strongly implicating sexual selection in speciation (West-Eberhard 1983; Ritchie 2007; Servedio and Boughman 2017). Male sexual signals and female preferences for those signals are often coupled (Butlin and Ritchie 1989; Boake 1991; Gray and Cade 1999) and even colocalized within the genome (e.g., Shaw and Lesnick 2009), which means that signal change could quickly contribute to reproductive isolation if preferences change in step. Yet the role of sexual selection in speciation remains controversial (Bolnick and Kirkpatrick 2012; Safran et al. 2013; Servedio and Boughman 2017), in part because observations of contemporary signal change and its consequences for sexual isolation are incredibly rare (Svensson and Gosden 2007). We discovered a novel sexual signal that we hypothesize resulted from selection on vestigial traits following sexual signal loss. Trait loss leaves the ghosts of traits past, remnants of behaviors, structures, and neural mechanisms that can be selected upon for different functions, providing a mechanism for the evolution of novelty (Walker-Larsen and Harder 2001; Carter et al. 2011). We know little about how novel sexual signals arise, but existing work suggests inadvertent cues resulting from mutations or cues previously not associated with mating could be refined into signals through coevolution between males and females, shaped by receiver sensory capabilities and preexisting biases (Ryan and Rand 1993). Here we characterize a new auditory signal in a cricket, showing that the sound differs from the ancestral signal in key characteristics that females use to locate conspecifics and assess mate quality. Further, we demonstrate that females can use the new sound to locate mates. Our discovery provides an unprecedented opportunity to watch the emergence of a new sexual signal unfold in real time.

Male crickets produce their sexual signal, song, using specialized wing structures (fig. 1*C*). Songs function in both species identification and mate choice (Otte and Alexander 1983; Otte 1994). Males use a loud calling song to attract females from long distances and switch to a lower-intensity courtship song during one-on-one interactions with females. Heterospecific songs differ in spectral characteristics (e.g., frequency, intensity) and temporal patterns, and are the major premating isolating barrier in several groups (e.g., Mendelson and Shaw 2002). The Pacific field cricket, *Teleogryllus oceanicus*, is native to northern and eastern Australia and typically produces a calling song with a dominant fundamental frequency of 4–5 kHz and a complex temporal pattern that consists of a long chirp followed by multiple short chirps (crickets who create this song are hereafter referred to

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Figure 1: Key structural forewing and song differences of representative lab-reared males from the silent flatwing Kaua'i population (A), purring Moloka'i population (B), and a normal-wing calling population (C). Sound is produced when males rub their forewings together, bringing the scraper of one forewing in contact with the teeth of the file (a modified wing vein) on the second forewing. The vibration-generating structures (file and scraper) are highlighted in red, and resonating structures (harp and mirror) are in yellow and blue, respectively. Purring males have reduced forewing-calling structures relative to typical calling males, while forewing structures of silent flatwing males are even more severely reduced or absent. Song pitch (frequency) and amplitude depend on size and shape of key wing structures and rate of stridulation. Detailed morphometric wing analyses are ongoing. Waveforms (*top*) and spectrograms (*bottom*) depict amplitude ($k\mu$) and frequency (kHz) of the calling song over time (s). The *Y*-axes are on the same scales across island populations (A-C) to facilitate comparison. Colors on the spectrograms indicate intensity. Purring song (B) is less intense than calling song (C) and differs visibly in spectral characteristics.

as typical; Balakrishnan and Pollack 1996). There is extensive intraspecific variation in the T. oceanicus calling song, suggesting the signal is evolvable, particularly in response to variation in natural enemies (Zuk et al. 2001). In a now textbook example (e.g., Dugatkin 2013; Molles and Sher 2018), Zuk et al. (2006) observed sexual signal loss in the Pacific field cricket. In two Hawaiian populations, the ability to produce song was lost remarkably quickly (12-20 generations on Kaua'i) in response to an introduced parasitoid fly (Ormia ochracea) that is attracted to cricket song and attacks singing males. Different single-gene mutations in each population cause a similar flatwing phenotype that eliminates the stridulatory and resonating structures on the wings (fig. 1A) that are used to produce and project the crickets' songs, resulting in silent crickets (Tinghitella 2008; Pascoal et al. 2014). The silent morph is protected from the parasitoid fly (Zuk et al. 2006), and preexisting behaviors (like satellite behavior and relaxed courtship requirements) and flexible behavioral strategies (like developmental plasticity favoring enhanced female phonotaxis) facilitate silent mate location (Bailey and Zuk 2008; Tinghitella et al. 2009) and courtship (Tinghitella and Zuk 2009). Flatwing crickets are the most complete example of signal loss ever observed. With our new discovery, we now have the opportunity to build on this work with a new set of questions about the origins of new sexual signals.

We discovered a population of *T. oceanicus* at the Kalaupapa National Historical Park (Kalaupapa) on Moloka'i, Hawaii, that contains silent males and males that produce a new call different from any described cricket call of which we are aware (supplementary audio files 1–3 and 7–9; all audio files are available online). The new song is reminis-

cent of a cat's purr (males who create this song are hereafter referred to as purring males). Wing morphology of purring males appears intermediate to that of typical and silent (flatwing) males (figs. 1, A1), and these differences persist in common-garden laboratory-rearing conditions (R. M. Tinghitella and E. D. Broder, unpublished data). Because the Kalaupapa population is a mix of purring and silent males, the newly evolved song may have resulted from selection on vestigial calling morphology and neural song patterning present in the flatwing phenotype. However, both silent (flatwing) and typically calling crickets are found in the Hawaiian Islands, so the ancestors of purring crickets could also have been typical callers. Distinguishing between the alternatives is beyond the scope of this work. On Moloka'i, T. oceanicus are found only on Kalaupapa and nowhere else on the island (unpublished data). We do not know whether parasitoid flies are present in Kalaupapa, though they are present 45 km away on O'ahu. On other islands, there appear to be consequences for the silent male phenotype when the frequency of calling males is low-the primarily (>95%) silent flatwing population on Kaua'i has experienced recent reductions in abundance (unpublished data). If the purr aids in mate location or courtship, the evolution of purring male crickets may be an example of evolutionary rescue, whereby populations experiencing stress avoid extinction through rapid adaptation (Bell 2017). In this study, we (1) test whether calling and courtship songs produced by purring males differ in key spectral characteristics compared to songs from a typical ancestral population, (2) determine whether the new purring song is a signal that female T. oceanicus can hear and use in long-distance mate location, and (3) assess the role of the purring song in short-distance courtship contexts.

Methods

Study Populations

We collected 47 adult (23 female) Teleogryllus oceanicus on the lawns of the Kalaupapa National Historic Park on Moloka'i, Hawaii, in May 2017. We allowed these females to lay eggs in multiple pads of moist cotton in the field, and then we shipped the egg pads to the University of Denver. We compared the calls of the F1 Moloka'i purring population to those of a typical ancestral population that was established in 2014 from animals collected at the University of California at Berkeley's Gump field station on Mo'orea, French Polynesia. Crickets from both populations were reared in Percival incubators (model I36VLC8) set to 25.5°C on a 12L:12D photoperiod inside of 1.9-L plastic containers in mixed-sex groups of 5-10 individuals. We provided ad lib rabbit food, egg carton shelters, and water from moistened cotton. Prior to the penultimate molt, when sex could be determined, we housed males individually in 0.5-L deli cups and females in groups of 5–10 in 1.9-L plastic containers. Moloka'i purring crickets were acoustically isolated from typically singing crickets until testing.

Characterizing the New Song

We recorded the long-distance calling and close-proximity courtship songs of first-generation, lab-reared purring adult Moloka'i males and 8th-10th generation, lab-reared, typically calling males from Mo'orea (calling song: N = 25 purring males and six typical males; courtship song: N = 21purring males and eight typical males). All recordings were done in an acoustically isolated professional recording studio using a Sennheiser MKH800 microphone set to the omnidirectional pattern. A Millenia HV-3D preamplifier was used with gain set to 48 dB. Inputs were recorded through an Avid HD analog-to-digital converter with a sampling rate of 192 kHz at 24-bit depth. We recorded males at a set distance (40 cm) from the microphone, allowing us to extract information about amplitude from the recordings. Anecdotal observations in the field suggested that the purring and typical songs differ in spectral characteristics (frequency and amplitude) but perhaps not in the temporal arrangement of pulses of sound (fig. 1). Thus, this first investigation of the purring song focuses on peak frequency, amplitude, and bandwidth. We imported WAV files into Audacity (http:// audacityteam.org) and filtered out ambient background noise using the noise reduction function. We then extracted the peak frequency and amplitude of that peak (relative intensity in decibels) from the first complete song of the first bout of continuous calling in each males' recording and measured the bandwidth 10 dB below peak frequency using the plot spectrum function in Audacity (settings: Hanning window, size = 2,048, log frequency axis).

Does Purring Song Facilitate Long-Distance Mate Location?

Because this is a newly discovered population of *T. oceanicus* with a novel song, variation in purring song and female preferences have not been characterized. We worked with first-generation, lab-reared purring males throughout this experiment. To produce the calls used in phonotaxis trials, we chose long-distance calling song recordings of five purring and five typical males. From each recording, we selected 10 sequential songs (~10 s) from the first recorded bout of continuous calling. The starting point for those 10 sequential songs within the bout was determined using a random number generator. We combined the 10 songs of each of the five purring males into a single WAV file that was looped during broadcast in phonotaxis trials. The phonotaxis recording of typical calling males was produced in the same way. Each female heard the songs in an identical order.

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To determine whether the purring song could be used in mate location (providing an advantage over silent males) and how it compares to the typical calling song in this regard, we used phonotaxis trials. We tested females from Moloka'i (purring population, N = 25) twice—once with the purring song and once with the typical song, conducted in random order with a 24-h break between trials. We isolated females for 2 days prior to the first phonotaxis test and between the two tests in their individual 0.5-L deli cups. Females were 7-14 days posteclosion at the time of testing, and we conducted all trials during the crickets' scotoperiod (darkness). Phonotaxis tests took place in a square arena (1.45 m²) built inside the acoustically isolated professional recording studio. The room was illuminated with two red lights and set to 25°C (range: 22.8-28.4°C, mean: 25.3°C). In each trial, we placed a female at the center of the arena under an inverted plastic cup for 2 min. We then simultaneously released the female and projected either the typical or purring song (randomly assigned) at biologically realistic amplitudes. EcoXBT wireless speakers were positioned in each corner of the arena, and the broadcasting speaker was randomly determined for each female to avoid position effects. One meter from the emitting speaker, the amplitude for looped typical songs was 64-78 dB, while the amplitude for the looped purring songs ranged from 52-62 dB. The following day, we retested each female using the same methods but with the alternative song type. We recorded the latency (time until first movement), search time (the difference between latency to first movement and the time until the female contacted the speaker), whether silent speakers were contacted, and if so, when. Trials lasted 5 min or until the female contacted the broadcasting speaker.

Does Purring Song Improve Male Performance in Courtship Interactions?

To determine whether purring song provides an advantage over silence in one-on-one courtship interactions, we tested Moloka'i females in standardized, no-choice courtship trials with intact and silenced Moloka'i males. Each female was tested with a unique male from each treatment (unmanipulated and silent) in random order. To silence males, we carefully removed the right forewing using dissecting scissors. Intact males were handled similarly but without removing the wing. At this time, we also measured male pronotum width, which we included as a covariate in our analyses. We conducted two sets of courtship experiments. In the first, both males and females were virgins, and these were the same females used for the phonotaxis experiment in the previous week (N = 25). Because female mounting rates were low in the first experiment and we hypothesized that this was due to lack of courtship and mating experience, we conducted a second experiment with experienced adults that were isolated from a mixed-sex colony for 4 days prior to the experiment (N = 15 females). For both experiments, each female was randomly paired with a male from one of the treatments (unmanipulated or silenced) in a 0.5-L deli cup with a clean piece of filter paper. We conducted experiments in an acoustically isolated, temperature-controlled room set to 25°C and illuminated with two red lights. We observed each pair for 10 min after they made first contact and recorded whether the male stridulated and whether the female mounted. We defined mounting (acceptance of the male mate) as presence of the female on top of the male for at least 2 s. For males in the silenced treatment, we could still observe stridulation when they lifted and moved the one intact wing. In each experiment, each female participated in a second courtship trial with a randomly selected male from the opposite treatment (unmanipulated or silenced) ~24 h after the first trial (experiment 1) or ~2 h after the first trial (experiment 2).

Analysis

To compare peak frequency, bandwidth, and amplitude of calling and courtship songs between purring and typical male songs, we used t-tests with unequal variances when appropriate. For phonotaxis experiments, where females could choose among a purring speaker and three silent speakers, we compared the number of females that first reached a purring speaker to the null expectation and calculated the probability of observing this result using a binomial test. To compare female responses to the purr and the typical call, we included only females that reached both speakers (N = 12), ensuring that these individuals could hear and respond to both signals. We calculated the difference in latency to movement (seconds) and search time (seconds) in response to purring song and typical song by subtracting values for each female (purr - call) and then comparing these values to the null, 0, in two one-sample, two-sided t-tests. For example, female Molo106 spent 28.1 s searching for the calling speaker and 96.9 s searching for the purring speaker. Thus, the difference in search time for female Molo106 was 68.8 s. All song and phonotaxis statistics were performed in JMP Pro 13.0.0.

For courtship trials, we used generalized linear mixed models with a binomial distribution since the response variable, female mounting, was yes or no. We modeled the two experiments separately. In both models, we included treatment (cut or unmanipulated), stridulation (yes or no), and the interaction between these two effects. We also included female ID as a random effect since females were each used in two courtship trials and included a measure of male size (pronotum width) as a covariate. We compared the full models to reduced models using χ^2 tests (performed in Ime4; Bates et al. 2017) in R, version 3.4.4, using the anova function (RStudio, ver. 1.1.442; R Core Team 2013), following

Winter (2013). When testing the interaction effect, the reduced model contained all model effects but the interaction, and when testing the main effects, the reduced model included the second main effect, the random effect, and all covariates. All data are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.gh7v525 (Tinghitella et al. 2018).

Results

Purring and typical males have calling and courtship songs that differ in key spectral characteristics. For long-distance calling song, the median peak frequency was 10.2 kHz (interquartile range [IQR] = 6.7-16.6 kHz) in our purring population, while the median peak frequency for the typical calling song was 4.9 kHz (IQR = 4.8-5.1 kHz; t = 5.32, df = 24.1, P < .0001, assuming unequal variances; fig. 2A). For courtship song, the median peak frequency for purring males was 7.6 kHz (IQR = 6.5-16.6 kHz) and for typical males was 5.0 kHz (IQR = 4.8-5.1 kHz; t = 3.86, df = 17.1, P = .0013, assuming unequal variances; fig. 2C). The calling and courtship songs of purring males were also more broadband than the ancestral calling song, with power distributed over a wider range of frequencies (fig. 2B, 2D). For calling song, the median bandwidth 10 dB down from peak frequency for purring males was 2.1 kHz (IQR = 1.3-4.6 kHz) and for typical males 0.5 kHz (IQR = 0.5-0.6 kHz; t = 6.29, df = 24.7, P < .0001, assuming unequal variances). For courtship song, the median bandwidth 10 dB down from peak frequency for purring males was 6.6 kHz (IQR = 4.4-10.6 kHz) and for typical males 0.4 kHz (IQR = 0.4-0.6 kHz; t = 7.68, df = 17.1, P < .0001, assuming unequal variances). Song amplitudes (measured at peak frequency) also differed for purring and typical males; the calling and courtship songs of purring males were lower in amplitude than those of typical males (calling: t = 19.21, df = 8.1, P < .0001, assuming unequal variances; courtship: t = 19.33, df = 17.7, P < .0001, assuming unequal variances).

To determine whether females could locate purring Kalaupapa males by their calling song, we compared the first speaker contacted for 25 females in phonotaxis trials with one calling (purring) speaker and three silent speakers. One female never moved and was not included in analyses (N =24). If the purr was no better than silent, we would expect females to contact a purring speaker 25% of the time and a silent speaker 75% of the time. Females were more likely to contact the purring speaker (N = 12) than a silent speaker (N = 5), and this differed significantly from the null expectation (fig. 3*A*; one-sided, P < .0001). To compare the efficacy of the purr to the ancestral calling song, we compared latency to movement and search time for those females that reached both the calling and purring speaker and for whom we had latency data (in subsequent trials, N = 12). Females began moving more quickly in response to a typical call compared to the purr (t = 2.24, df = 11, P = .05) and spent more time searching for the purring speaker compared to the ancestral call (fig. 3*B*; t = 4.08, df = 11, P = .0018).

In courtship trials, we did not find evidence that purring affected female mounting. In both experiments, neither male treatment (unmanipulated or silenced), regardless of whether the male stridulated, nor the interaction between treatment and stridulation predicted female mounting (table 1). In the first experiment with virgin males and females, 16 out of 50 courtship trials ended in mounting, and in 10 of those 16 trials, females mounted in the absence of stridulation. Five females mounted both the unmanipulated male and the silenced male, five females mounted only the unmanipulated male, and one female mounted only the silenced male. In the second experiment with experienced males and females, there was a trend for stridulation to affect mounting (P = .06). However, only two of 15 females mounted in this experiment, and both mounted in the absence of stridulation, which is in the opposite direction to what we would expect if purring (or stridulation) positively elicited mounting.

Discussion

We discovered a new morph of male Teleogryllus oceanicus in the Hawaiian Islands that has a novel song and wing morphology (fig. 1). The purring song differs in frequency and amplitude from the typical ancestral calling song, and it is more broadband (fig. 2). Purring song functions as a signal that some females can detect, and it provides advantages over silence in mate location (fig. 3). However, the new signal does not appear to play a role in courtship behavior (table 1), though it is important to note that mounting rates were quite low in the current study, so we interpret our courtship results with caution. Faced with environmental change, organisms can persist and flourish through rapid evolutionary responses, phenotypic plasticity, or a combination of the two. Teleogryllus oceanicus introduced to the Hawaiian Islands have undergone remarkably fast evolutionary change in response to a parasitoid fly, Ormia ochracea, they encounter nowhere else in their range (Zuk et al. 2006; Pascoal et al. 2014). Males on the islands of Kaua'i and O'ahu lost the ability to communicate with mates via song over ~12-30 generations. Our latest discovery in this system, purring males, offers an opportunity to better understand the mechanisms by which evolutionary novelties arise and coevolve between the sexes.

The greatest challenge that silent or mostly silent *T. oceanicus* populations face is long-distance mate location without song. Silent flatwing males on Kaua'i act as satellites to the remaining calling males in the population, facilitating mate location (Zuk et al. 2006), and the tendency to use sat-



Figure 2: Frequency histograms showing peak frequency (kHz) of the calling songs (A) and courtship songs (C) of purring males (black filled bars) and typical males (open bars). Boxplots are shown above frequency histograms and depict the minimum, 0.5%, 2.5%, 10%, 25%, 50%, 75%, 90%, 97.5%, and maximum quantiles for typical (*top*) and purring (*bottom*) male peak frequencies. Power spectra of representative purring (solid lines) and typical (dashed lines) long-distance calling (B) and courtship (D) songs. Each line represents the song of a single male (supplemental audio files 1–12).

ellite behavior predates the flatwing mutation (Tinghitella et al. 2009). However, in 9 nights of sampling on Kaua'i in 2014, 2015, and 2017, we heard no calling males (R. M. Tinghitella, personal observation). Without at least a small proportion of calling males, satellite behavior is unlikely to solve the problem of locating mates without song. In 9 nights of sampling in Kalaupapa, we heard no calling males, and none of the >1,000 lab-reared offspring of 35 field-caught Kalaupapa females were capable of producing the typical song. We observed some Kalaupapa males stridulating dur-



Figure 3: *A*, We report the first speaker contacted for the 17 Kalaupapa females that made contact with a speaker during phonotaxis experiments (black bars), in which speakers emitted prerecorded male calling song from purring Kalaupapa males (purr) or mimicked silent males and emitted no sound (silent). The expected encounter rate (gray bars) is three times higher for silent speakers than purring speakers. *B*, For females that reached the speaker broadcasting typical ancestral calling song as well as the speaker broadcasting the purr (N = 12), we report the mean (±SE) difference in female response (latency to movement toward purr – latency to movement toward normal call) and the mean (±SE) difference in total search time (search time to reach purr – search time to reach normal call).

ing recording sessions but producing no audible sound, suggesting they had flatwing morphology. The presence of silent stridulating males in the Kalaupapa population leads us to hypothesize that the purring phenotype evolved from flatwing silent males via selection on vestigial behavior, morphology, and neural pathways that persisted following the flatwing mutation. In our phonotaxis experiment, 58.3% of females contacted the purring speaker, thus offering an advantage over silence in terms of mate location. Females may be preadapted to orient to songs in this frequency range (median 10.2 kHz in the current study) since there is a population of auditory receptor fibers responsive to frequencies near 10-12 kHz (Imaizumi and Pollack 1999). Our design, however, does not allow us to distinguish between an advantage of the purring calling song, specifically, versus any sound in that frequency range. Future work should test the efficacy of the purr versus sounds with similar spectral qualities and even white noise. Additionally, because the purr is so broadband, the peak frequency that we identified was often very similar in amplitude to other peaks within an individual male's song. It may therefore be important to explore the importance of a series of frequencies across the range of female cricket hearing for phonotaxis and courtship song.

We found no evidence that purring courtship song or stridulation of silenced or unmanipulated males affected female mounting rates in this initial lab experiment. However, anecdotally, mounting rates appear to be higher in the field than in the lab; in preliminary courtship trials conducted in the field with purring males of unknown age and mating status, females mounted ~50% of unmanipulated males. It will be important to explore courtship behavior across generations and in the field as well as the lab. Females in silent populations of *T. oceanicus* accept the mating advances of silent males who do not produce the ancestral call (Bailey et al. 2007; Tinghitella and Zuk 2009), and females from the purring population may thus be preadapted to mate with purring males who lack the typical courtship signal. Further, being reared in silence renders females more responsive to less preferred calling songs (Bailey and Zuk 2008), so plasticity may further reduce mating requirements in our purring population. While acoustically isolated from typical males, females in our study could hear purring males during development, and we do not know what effect this has on female mating decisions.

The parasitoid fly *O. ochracea* played an important role in the loss of calling ability in this system (Zuk et al. 2006) and may be critical in the evolution of this novel signal. *Ormia ochracea* is found on all other Hawaiian Islands where

Table 1: Generalized linear mixed models testing effects of treatment (unmanipulated or silenced), stridulation, and their interaction on female mounting in experiments with virgin animals (experiment 1) and nonvirgin animals (experiment 2)

Experiment, model effect	χ^2	df	Р
1. Virgins:			
Treatment	1.28	1	.26
Stridulation	1.75	1	.19
Treatment × stridulation	1.77	1	.18
Pronotum width	1.02	1	.31
2. Nonvirgins:			
Treatment	1.80	1	.18
Stridulation	3.42	1	.06
Treatment × stridulation	.00	1	1.00
Pronotum width	5.07	1	.02

the cricket is found (including O'ahu, 42 km from Moloka'i), and the fly's hearing ability is closely tuned to the frequency of the ancestral cricket call (fly tuning 4-6 kHz [Robert et al. 1992] and typical T. oceanicus song 4-5 kHz [Balakrishnan and Pollack 1996]). The peak frequency of the new purring long-distance song falls well outside of this range (median 10.2 kHz), and the purr is lower amplitude than the typical call, however, most purring songs do contain sound of around 5 kHz with a low relative amplitude (J. D. Wilson, unpublished data from calling song frequency analysis conducted in Audacity). The purring call appears to fall within the range of hearing for female T. oceanicus (Imaizumi and Pollack 1999), and a subset of our females were phonotactic to the new sound. If the fly is present on Moloka'i but cannot hear the purring song, the Moloka'i population may have evolved a private mode of communication that facilitates mate location but prevents parasitism (Reichard and Anderson 2015). We are currently investigating this hypothesis as well as exploring temporal and harmonic components of song, since these elements should affect female mating decisions (Balakrishnan and Pollack 1996).

Several intriguing questions remain. The mutations to the flatwing phenotype on Kaua'i and O'ahu occurred within a few years of one another and are morphologically and genetically distinct (Pascoal et al. 2014). Two alternative hypotheses for the origin of purring males are possible: the purring phenotype we found on Moloka'i may result from yet another mutation that reduces conspicuous sexual signaling (on a typical calling male genetic background), or it might represent selection on vestigial calling morphology and neural song patterning (Schneider et al. 2018) of flatwing males if Moloka'i was colonized by crickets from another Hawaiian Island where flatwings are found. Further, we do not know whether the purring phenotype is related to parasitism by *O. ochracea*, whether the flies are present on Moloka'i, whether they can hear the purr, or whether the purring phenotype protects males from the parasitoid. Finally, speciation is believed to be tied to the evolution of novel communication systems. There is growing appreciation for the role of sexual selection in speciation, but comparisons are necessarily made between closely related species or populations that already differ in sexual signals. Our discovery of purring crickets is an unprecedented opportunity to observe the origin of an entirely new sexual signal that differs from any known cricket song and to explore the coevolution of this signal with female preferences and natural enemies.

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APPENDIX

Wing Morphology of Typical, Purring, and Silent Male Teleogryllus oceanicus



Figure A1: Photos of the right forewing for representative males of the three types: typical calling male (*A*), Moloka'i purring male (*B*), and derived silent male (*C*).

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Left, a purring male from Moloka'i. Right, a purring male singing to attract mates. Photo credit: E. Dale Broder.