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Original Article

Varied female and male courtship behavior facilitated the evolution of a novel sexual signal

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Sexual selection can contribute to speciation when signals and preferences expressed during mate choice are coupled within groups, but come to differ across groups (generating assortative mating). When new sexual signals evolve, it is important to investigate their roles in both mate location and courtship contexts, as both signaling functions are critical in mate choice. In previous work, researchers identified two new male morphs (silent and purring) in Hawaiian populations of the Pacific field cricket, *Teleogryllus oceanicus.* These morphs likely evolved because they protect males from an acoustically orienting parasitoid, yet still obtain some reproductive success. But, it remains unknown how the purring morph functions in close courtship encounters. We compared the relative success of the very recently evolved purring morph to that of the ancestral and silent morphs during courtship encounters. Purring males produce a novel courtship song and were not as successful in courtship as the ancestral type, but were mounted by females as often and as quickly as the obligately silent morph that arose and spread ~20 years ago. Purring males initiate courtship more quickly than other morphs, and females from populations where purring is common exhibit higher overall mounting rates. Thus, differences in the behavior of purring males and of females from populations where purring is common may have facilitated the origin of this novel sexual signal. We found no assortative mating between males of a given morph and females from their own population, and so we hypothesize that multiple male types will be maintained within the species because each achieves fitness in different ways.

Key words: **evolutionary novelty, flatwing, purring cricket, rapid evolution, *Teleogryllus oceanicus*.**

INTRODUCTION

Animals communicate with one another using sexual signals during mate location and courtship. Many closely related species differ most obviously in these signals, suggesting that sexual selection is important in the generation and maintenance of biodiversity (West-Eberhard 1983; Servedio and Boughman 2017). For instance, Hawaiian swordtail cricket species in the genus *Laupala* are ecologically indistinguishable from one another, but produce distinct songs that are preferred by females from their own species; this coupling of signals and preferences within species prevents heterospecific matings (Mendelson and Shaw 2002). When sexual signals change or new signals arise, then, it is especially important to understand how they function in courtship because this will inform our understanding of how sexual selection contributes to the origins and maintenance of biodiversity (Broder et al. 2021a).

Sexual signals are conspicuous traits that evolve in response to a variety of evolutionary forces and ecological circumstances including natural enemies, competing signalers, sensory environments, mate availability, and the preferences of potential mates (e.g., Endler 1980; Zuk and Kolluru 1998; Yeh 2004; Fowler-Finn and Rodríguez 2012; Head et al. 2013). One particularly prominent selection pressure acting on sexual signals is eavesdropping natural enemies. When predators and parasites exploit sexual signals to locate prey and hosts (Zuk and Kolluru 1998), signals and signaling behavior can evolve to reflect an evolutionary compromise between selection imposed by mates (intended receivers) and selection imposed by natural enemies (unintended receivers). Such is the case for male túngara frogs (*Physalaemus pustulosus*) whose calls are eavesdropped upon by frog-eating bats (*Trachops cirrhosus*) (Akre et al. 2011), and male guppies (*Poecilia reticulata*) whose coloration evolves in response to preferences of both female conspecifics and predatory fish (Endler 1980). Conflicts between natural and sexual selection frequently result in plastic and evolutionary changes in signaler and receiver traits within species (Rand and Ryan 1981; Belwood and Morris 1987; Lewkiewicz

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and Zuk 2004), and over the long term could also drive divergence and even speciation.

Our current understanding of the mechanisms by which sexual signals diverge and the consequences for pre-mating isolation is limited by the rarity of opportunities to directly watch that process in real time (Broder et al. 2021a). What we know comes largely from studies comparing closely related species that already differ in sexual signals and have for great periods of time. Rapid evolution, which operates on timescales directly observable to researchers and is often spurred by anthropogenic change (Reznick and Ghalambor 2001; Carroll 2007; Sih et al. 2011), may offer opportunities to directly observe the process of signal divergence, although the rapid evolution of sexually selected traits still appears to be relatively rare (Svensson and Gosden 2007; Svensson 2019). The very recent evolution of a novel sexual signal in the Pacific field cricket, *Teleogryllus oceanicus* (Tinghitella et al. 2018a), thus offers a rare opportunity to investigate how males with recently evolved novel sexual signals fare in the context of close one-on-one courtship encounters.

Originally endemic to Australia and the South Pacific, *T. oceanicus* colonized the Hawaiian Islands sometime before 1877 with the help of humans (Otte and Alexander 1983; Zhang et al. 2021). In Hawaii, *T. oceanicus* experiences selection from a deadly eavesdropping parasitoid fly that it encounters nowhere else in its range. The parasitoid fly, *Ormia ochracea*, is also introduced to Hawaii, having expanded their range from North America to the Hawaiian Islands sometime before 1989 (Evenhuis 2003). Gravid female *O. ochracea* locate male crickets by exploiting the loud songs the crickets produce to attract females. After locating a cricket host, the parasitoids larviposit on and around the male cricket, and larvae then burrow into the host where they feed on host tissues until pupating around 10 days later (Cade 1975).

Much recent evolutionary change in the songs that male *T. oceanicus* produce has been attributed to selection to avoid parasitism (Zuk et al. 2006; Tinghitella 2008; Pascoal et al. 2014; Tinghitella et al. 2018a, 2021). Completely silent male morphs ('flatwing') evolved twice in quick succession on the islands of Kaua'i and Oahu about 20 years ago (Zuk et al. 2006; Pascoal et al. 2014). More recently, males producing a new, novel song, called 'purring' were discovered (Tinghitella et al. 2018a). The songs of purring males sound superficially like a cat's purr, are distinct in frequency, amplitude, and bandwidth from those produced by the ancestral type (hereafter 'typical'), and are produced using changed wing morphology (Tinghitella et al. 2018a, 2021). Purring males were discovered on the island of Moloka'i at Kalaupapa National Historical Site, but are now found in several other Hawaiian populations and are increasingly common in our biannual sampling (Tinghitella et al. 2021). Like silent crickets, purring males appear to be protected from fly parasitism (Tinghitella et al. 2021), which may explain the morph's rapid spread.

Male crickets produce two songs in the context of intersexual mating interactions. The first is a loud, long-distance calling song that attracts females from afar. Once the female approaches, males begin producing a second, softer, close-range courtship song (Alexander 1961). This implies two potential barriers to mating: males must first successfully attract a mate from a far distance and second, entice the attracted female to mount. Female *T. oceanicus* overwhelmingly prefer typical male calling songs from longer distances, but can also use purring calling songs to locate mates (Tinghitella et al. 2018a), and purring outperforms white noise and silence in this regard (Tinghitella et al. 2021). How the novel purring signal fairs in the context of one-on-one close range courtship, however, has not yet been investigated. A purring male who attracts a female from afar or encounters one randomly, but cannot convince her to mate, still has zero fitness. Further, when songs change we might expect male and female courtship behaviors to change as well. Courtship often involves the exchange of several integrated and sometimes reciprocal signals and behaviors between males and females. Males with novel or less preferred mating signals may need to use different sequences of behaviors to elicit favorable female responses (e.g., Boughman 2001; Tinghitella and Zuk 2009; Tinghitella et al. 2018b), may be more successful than the ancestral type if novelty per se is preferred, or may be less successful overall than males with ancestral signals.

Here, we determine the relative courtship success of typical, purring, and silent male morphs of *T. oceanicus* and examine whether each achieves reproductive success using the same or different courtship strategies. We paired males of the three morphs in courtship trials with females from the same three island populations (Figure 1) in a fully factorial design. Each population studied differs in evolutionary history (e.g., the extent to which and for how long they have encountered silent, purring, and typical males). Our design thus allowed us to ask if success in courtship and specific courtship behaviors depend on male morph, the female receiver's population (evolutionary history), or the interaction of the two. We used animals from Hilo (Big Island of Hawai'i), Kalaupapa (Moloka'i), and Wailua (Kaua'i) in this study. Hilo overwhelmingly contains typical males (<2% silent, 0% purring; Pascoal et al. 2014 and personal observation), Kalaupapa only contains purring males in our recent surveys (Tinghitella et al. 2018a, 2021), and Wailua historically contained >95% silent males (Zuk et al. 2018), but at present contains both silent and purring males (Tinghitella et al. 2021).

While field crickets are thought of as having a classically female choice mating system, males do have the ability to enact courtship decisions by choosing which females to court and how. We thus investigated behaviors indicative of female mating interest and decisions, plus behaviors indicative of male mating interest and courtship effort. Since purring and silent males produce attenuated or no song, we predicted that they may begin courting females more quickly and court less discriminately than the ancestral, typical type. But, we anticipated that typical males would be mounted more often and more quickly than purring males, who would be mounted more often and more quickly than silent males. We also predicted that females from populations where purring and silent males are more common (Kalaupapa and Wailua) would be more likely to mount these two male morphs than females from the population where they are largely absent (Hilo). Finally, we tested for assortative mating within types, because ultimately, divergent receiver responses during courtship can generate and maintain reproductive isolation between closely related organisms (Mendelson and Shaw 2002). Evidence for assortative mating by morph type would come from the highest rates of mounting occurring in homotypic pairings (between males of a particular morph and females from a population that largely contains that morph). Little time has passed since the evolutionary origin of the silent and purring male morphs, and relaxed female preferences have been a hallmark trait of Hawaiian *T. oceanicus* facilitating rapid evolutionary change in this study system (Bailey et al. 2008; Tinghitella and Zuk 2009; Zuk et al. 2018), so we expected to find little, if any, evidence for assortative mating. Whether or not we find assortative mating, understanding how courtship proceeds soon after novel sexual signals evolve will help uncover how such signals become established.

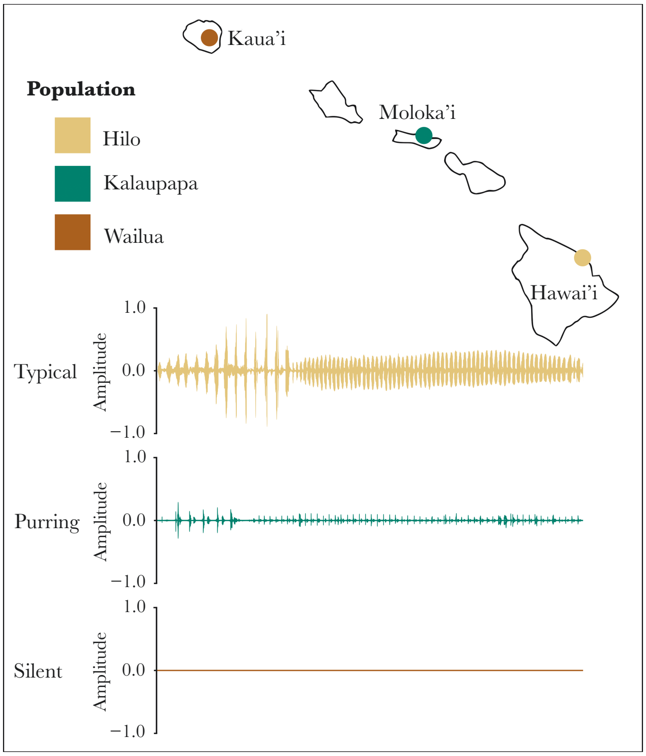

Figure 1
Hilo, Wailua, and Kalaupapa are three representative populations across the islands of Hawaii where the typical, silent, and purring male morphs, respectively, predominated at the time of the experiment. The morph composition of these populations is highly dynamic and determining precise morph compositions requires extensive collection, morphological analysis, and song recordings and analysis. Here, we show representative waveforms for male courtship songs produced by each morph; each audible song consists of a chirp followed by a long trill. The three male morphs differ in calling and courtship song characteristics, as well as their underlying wing structures (Tinghitella et al. 2018a).

METHODS

Collection and rearing

We collected cricket eggs from field-caught adult females in 2018 and 2019 at three locations across Hawaii that contain three prominent male morphs of the Pacific field cricket (Figure 1). We brought eggs from each of these field collection sites back to the University of Denver and established lab colonies for each (hereafter "population"). The studies described below took place between July 2019 and August 2020 with 2nd–6th generation lab-reared animals. Crickets in this experiment were reared inside of Percival incubators (model I36VLC) set to 25.5 °C on a 12:12 light:dark cycle. We housed juveniles inside of 15 L plastic containers (approximately 30 crickets per box) with moistened cotton, ad libitum food (Purina rabbit food for adults and Fluker's cricket chow for juveniles), and egg carton for shelter. To prepare them for courtship trials, we isolated virgin females individually in 0.5 L deli cups within 3 days of their final molt (eclosion), assigned them a unique ID, and recorded their eclosion date. On isolation, females were housed in incubators that contained males from their own population, so they were exposed to acoustic environments that mimicked what they would hear in nature.

Pre-courtship matings

To avoid the indiscriminate mate preferences that sometimes occur in virgin female crickets (Lickman et al. 1998), we ensured that females mated exactly twice prior to their use in a focal courtship trial (following Tinghitella and Zuk 2009). We paired each female with a randomly chosen male from their own population in a deli cup for 2 h per day for up to 8 days until they mated exactly twice. If a female did not mate twice in this time period, we omitted her from the experiment. Five Hilo females (the typical population), 21 Kalaupapa females (the purring population), and 45 Wailua females (the historically silent population) did not complete the pre-courtship matings. Interestingly, the number of females completing pre-courtship mating trials did differ across populations, with more females from Hilo completing pre-courtship matings overall ($X^2 = 22.194$, df = 2, $P < 0.001$).

Focal courtship trials

Our principal goal was to determine the relative success of different male morphs in courtship interactions and whether their mounting success depended on the home population (and thus evolutionary history) of potential female mates. Note that singing wing morphology is sex-limited (only males have the wing structures to produce song), so we cannot identify the genotype of females based on their phenotypes (Tinghitella 2008). We paired adult typical, purring, or silent males in standardized no-choice courtship trials with females from the same three island populations (Figure 1) who had completed pre-courtship matings. We used a fully factorial design for a total of nine possible trial types ($N = 211$). Females that completed pre-courtship matings were 16.5 ± 4.3 days post-eclosion at the time of focal courtship trials. The males in focal courtship trials were chosen at random from stock populations, and thus did not have known mating histories, but were isolated from females in 15 L plastic containers for at least 24 h prior to focal trials to encourage courtship singing. Silent and purring males have superficially similar wing morphology (Tinghitella et al. 2018a), so we determined the morph of Wailua males using a combination of visual observation of wing morphology and listening for the production of audible courtship song. Males that did not produce audible songs when stridulating actively were considered to be silent, as has been standard practice in this study system. Note that silent males are increasingly rare in the field (Gallagher et al. in review, personal communication), and accordingly, in our lab-reared populations. During focal courtship trials (when we were able to listen closely for song in one-on-one encounters) we found that 69% of Wailua males were silent and 31% were purring. We only used silent males from Wailua, purring males from Kalaupapa, and typical males from Hilo in focal courtship trials.

No-choice focal courtship trials were conducted in an acoustically-isolated room lit only with red light. For each courtship trial, we placed a female in a 0.5 L deli cup lined with filter paper. We gave the female 1 minute to acclimate before starting the trial by gently adding a male to the deli cup. If the male and female did not physically encounter one another within one minute, we gently nudged them together using the eraser end of a pencil. Males had five minutes from their introduction to the courtship deli cup to begin stridulating (lifting and rubbing their wings together). If a male did not stridulate in that time, we replaced him with another male up to three times. If no males stridulated, we removed the female from the experiment ($N = 26$ from Hilo, $N = 9$ from Kalaupapa, $N = 15$ from

Wailua). Female populations differed in the extent to which males stridulated during these trials ($X^2 = 7.5206$, df = 2, $P = 0.023$); a smaller proportion of trials were completed (successful stridulation) when males were paired with females from the Hilo population. Once the male stridulated, the trial continued for another 10 min, or until the female mounted him. If the female did not mount the male in this time, the trial was ended. Immediately after mounting, we gently separated the crickets to avoid cross-population mating and returned the animals to their respective lab colonies.

We recorded the presence of and latency to several behaviors that are indicative of male courtship effort (latency to stridulation defined as the time from introduction to the deli cup to first stridulation) and female interest and mating decisions (whether the female mounted the male provided to her and if so, her latency to mount from first stridulation to mounting). Occasionally, a female would mount a male without any prior stridulation ($N = 13$). In these instances, mount (yes or no) was recorded, but latency to stridulate and latency to mount were not. We took faster stridulation times to be indicative of greater male interest and faster mounting times to be an indication of female interest in males. We also noted additional behaviors that may be relevant to courtship outcomes, including whether or not the female was aggressive toward the male and whether the male was a consistent courter (if he stridulated for >50% of the trial from first stridulation to mount). Finally, we measured the size of the male and female (pronotum width measured using Vernier digital calipers to the nearest 0.01 mm).

Statistical analyses

We ran all statistics in R (R Core Team 2021) and constructed all data-based figures with the ggplot2 package (Wickham 2016). We first visualized q–q plots and identified the distribution that best fit the data using the DHARMa package (v0.4.4; Hartig 2021). Negative binomial distributions best fit the continuous variables latency to stridulation and latency to mount. We ran the remaining models (mounting success, aggression, consistent courting) with a binomial distribution (0/1 variables).

To determine whether different male morphs are more or less successful in courtship and whether that depends on the receiver (female population), we ran generalized linear models. For continuous variables with negative binomial distributions we used Template Model Builder with the package glmmTMB (Brooks et al. 2017). The basic model structure included male morph, female population, and their interaction as main effects. The male morph × female population interaction allowed us to test whether female responses to different male morphs depended on the population from which the female originated. Evidence for assortative mating would come from higher within population than between population mating rates, which would be captured in this interaction effect. In the latency to stridulate model, we included female pronotum width and female age as covariates, as such characteristics may affect male interest in courting them (Mautz and Sakaluk 2008). In the mounting success and latency to mount models, we included female age and male pronotum width as covariates, as both may affect female mounting decisions (Bateman et al. 2001; Mautz and Sakaluk 2008). The interaction between male morph and female population was never significant and therefore removed from all models, which allowed us to make post hoc pairwise comparisons using the emmeans package (v1.5.5-1; Lenth 2021) to identify which morphs or female populations differed in behavior.

During the courtship trials, we noticed that some males stridulated more consistently than others and some females were aggressive towards the males they encountered. We ran two additional models to investigate whether aggression and consistent courting were associated with male morph or female population. The aggression model structure was the same as in the mounting success and latency to mount models. The consistent courting model included male morph, female population, and their interaction as main effects, and female pronotum width, female age, and latency to mount as covariates. The latency to mount was included as a covariate here because trials differed dramatically in length, especially depending on male morph, and we were interested to know whether trial length was associated with whether a male courted consistently. In both models, the interaction of male morph and female population was not significant and was therefore removed from the model.

RESULTS

We first asked whether males begin courting females (latency to stridulation) more or less quickly depending on their own morph, the population of the female they were courting, or their interaction. Latency to stridulation was not associated with the population of the female, but the latency to stridulate did differ between male morphs (Table 1, Figure 2). Purring males began stridulating sooner than both silent males ($t = -3.335$, $P = 0.003$) and typical males ($t = -2.499$, $P = 0.035$), but typical and silent males did not differ ($t = 0.812$, $P = 0.696$).

Next, we asked whether mounting success or latency to mount varied across our courtship trial types. Whether a male was mounted or not depended on both the male's morph and population of the female he was paired with (Table 1, Figure 3A). Typical males were mounted in 85% of trials, more than both purring males (57%; $z = -4.395$, $P < 0.001$) and silent males (46%; $z = -4.561$, $P < 0.001$) overall. Purring and silent males were equally likely to be mounted ($z = -0.005$, $P = 1.000$). Interestingly, females from Kalaupapa where all males are of the purring morph, mounted more males overall than females from Hilo (the typical population; $z = -3.782$, $P < 0.001$) or Wailua (the historically silent population; $z = 3.681$, $P < 0.001$), but females from Hilo and Wailua did not mount at different rates ($z = -0.392$, $P = 0.919$). Latency to mount depended only on the male's morph (Table 1, Figure 3B). As expected, the latency to mount was shorter for typical males than it was for purring males ($t = 2.809$, $P = 0.016$) or silent males ($t = 2.725$, $P = 0.020$). Purring and silent males did not differ in how quickly they were mounted ($t = 0.026$, $P = 0.999$).

Finally, we looked at how female aggressive behavior and consistent courting by males were distributed across our courtship trial types. Whether males were consistent courters was associated with both morph and the population of the female they courted. Typical males consistently courted in only 36% of trials and were less consistent courters overall than silent males (62%; $z = 3.397$, $P = 0.002$), but did not differ from purring males (59%; $z = 2.053$, $P = 0.100$), and purring and silent males did not differ from each other ($z = -1.685$, $P = 0.2111$) (Table 1, Figure 4A). Females from Kalaupapa, where males are predominantly purring, were more consistently courted (73%) than those from the typical population (Hilo; 42%, $z = -2.546$, $P = 0.029$). Females from Wailua (49% courted) were no more or less likely to be courted than females from Hilo or Kalaupapa (Wailua vs Kalaupapa: $z = 1.291$, $P = 0.401$; Wailua vs. Hilo: $z = -1.413$, $P = 0.334$).

Table 1

Summary of generalized linear models investigating how male morph and female population impact courtship outcomes in *T. oceanicus*. For models with negative binomial distribution we report Chisq values and for models with binomial distribution we report LR Chisq values. Model parameters significant at $P < 0.05$ are bolded

Model name & type	Parameter	(LR) Chisq	Df	Pr(>Chisq)
Latency to stridulate				
Generalized linear model	Intercept	59.519	1	**<0.001**
Family: Negative binomial	Female location	2.212	2	0.331
	Male morph	11.745	2	**0.003**
	Female pronotum (mm)	0.672	1	0.413
	Female age (days post eclosion)	3.245	1	0.072
Mount				
Generalized linear model	Female location	19.934	2	**<0.001**
Family: binomial (logit)	Male morph	30.292	2	**<0.001**
	Male pronotum (mm)	0.371	1	0.543
	Female age (days post eclosion)	0.758	1	0.384
Latency to mount				
Generalized linear model	Intercept	26.64	1	**<0.001**
Family: Negative binomial	Female location	2.005	2	0.367
	Male morph	10.392	2	**0.006**
	Male pronotum (mm)	1.535	1	0.215
	Female age (days post eclosion)	0.26	1	0.61
Aggression				
Generalized linear model	Female location	2.512	2	0.285
Family: binomial (logit)	Male morph	17.542	2	**<0.001**
	Male pronotum (mm)	0.008	1	0.931
	Female age (days post eclosion)	0.154	1	0.695
Consistent courting				
Generalized linear model	Female location	6.821	2	**0.033**
Family: binomial (logit)	Male morph	15.053	2	**0.001**
	Female pronotum (mm)	0.009	1	0.926
	Female age (days post eclosion)	0.039	1	0.843
	Latency to mount (sec)	0.374	1	0.541

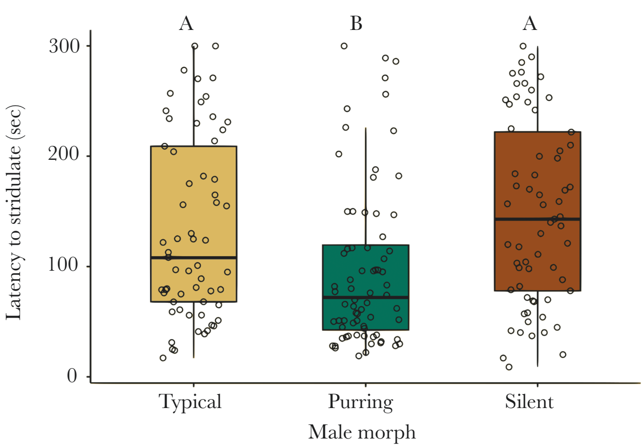

Figure 2

Latency to first stridulation varies across male morphs, with purring males initiating courtship stridulation more quickly than typical or silent males. Male morphs not labeled with the same letter differed significantly in latency to stridulate.

Whether females were aggressive toward males depended on the male's morph, but not the female's population (Table 1, Figure 4B). Females were less aggressive towards typical males (2% of trials) than they were to either of the more recently evolved morphs (purring, 24%, $z = 3.266$, $P = 0.003$; silent, 20%, $z = 2.618$, $P = 0.024$). Females were equally aggressive to silent and purring males ($z = 1.173$, $P = 0.469$).

DISCUSSION

We set out to compare the relative fitness of typical, silent, and purring *T. oceanicus* males in courtship, to discover how novel male morphs accrue fitness in courtship interactions, and to determine whether success or courtship strategy depends on the receiver's evolutionary history. Overall, typical males fared best in courtship (were mounted more often and more quickly), but purring males and silent males have roughly equivalent success in courtship, with about 50% of courted females mounting them. The three male morphs, however, seem to accrue their fitness using a different suite of signals and behaviors during courtship; males who lack the ancestral signal seem to achieve success differently (as in some other study systems: Tinghitella et al. 2015; Romero-Diaz et al. 2019). Despite having more aggression directed toward them, purring males initiated singing more quickly than the other two types and after initiating singing, silent males were more consistent courters than typical males. Taken together these results suggest the newer morphs may need to exert relatively high courtship effort before females will mount them. Finally, the behavior of females from Kalaupapa, where purring males predominate, may have facilitated the persistence of this novel morph and song type there; females from Kalaupapa mounted males at higher rates overall, regardless of the morph with which they interacted.

To achieve reproductive success, a male cricket must survive long enough to attract and court females (and in this case, avoid premature death to parasitism), locate mates (usually by attracting females from afar using calling song), and convince females to mount them once they are in close proximity (using courtship

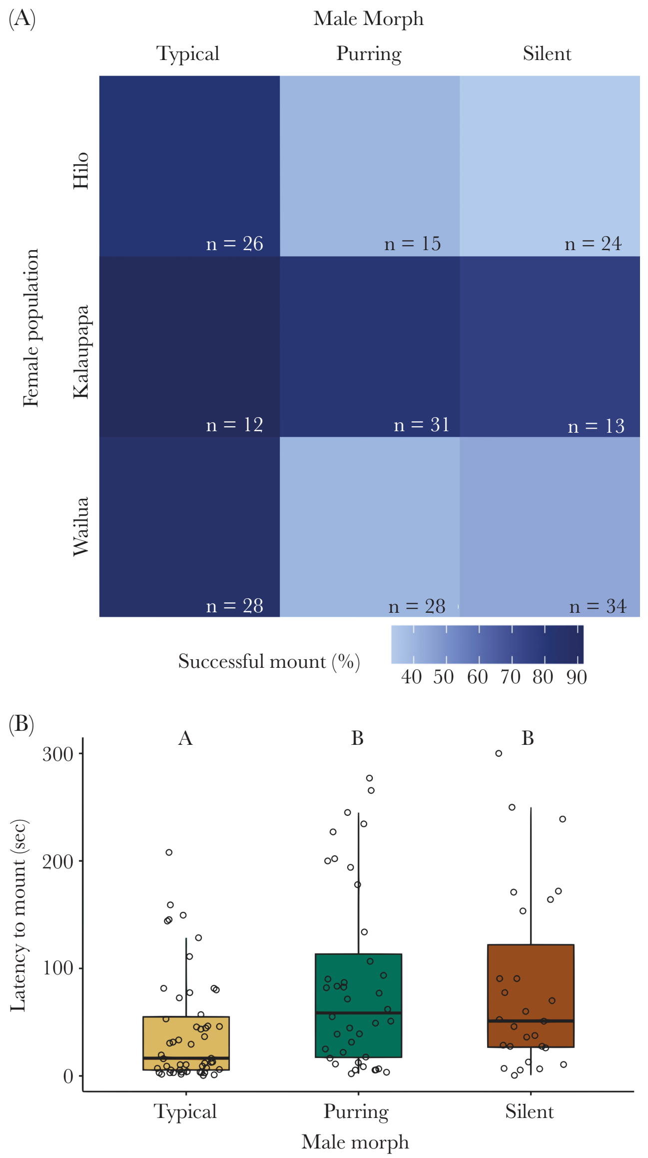

Figure 3
Mounting rates and latency to mount vary across male morphs and female populations. (A) As predicted, typical males were mounted more often than the novel male types (silent, purring) and Kalaupapa females (who are from a predominantly purring population) mounted males of all morphs at higher rates overall than females from other tested populations. (B) Typical males were also mounted more quickly than the other two types, consistent with our expectations. Male morphs not labeled with the same letter differed significantly in latency to mount.

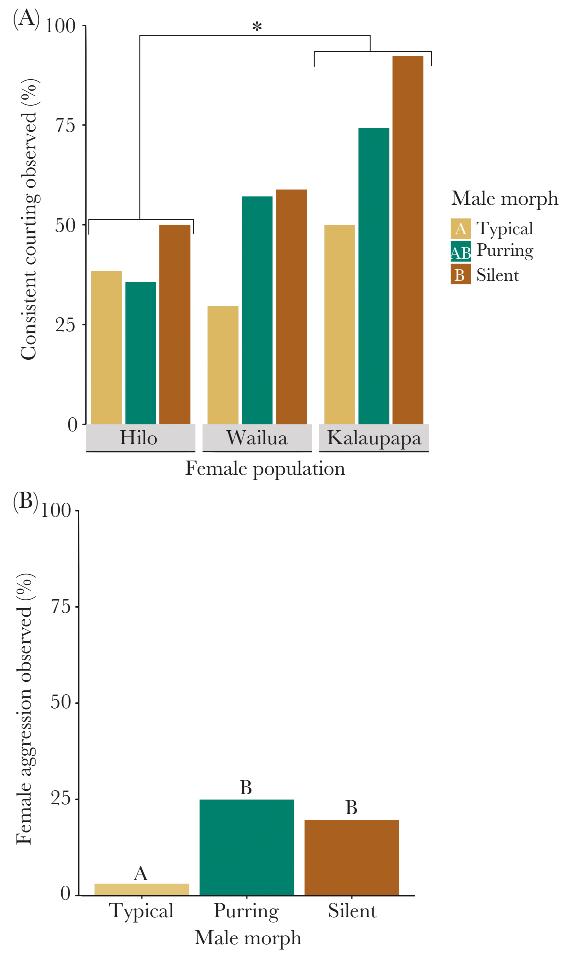

Figure 4
Consistent courting depended on both male morph and female population of origin and female aggression depended only on male morph. (A) Silent males courted more consistently than typical males, as indicated by the letters in the figure legend. Females from Kalaupapa (a predominately purring population) were courted more than those from Hilo as indicated by the * above the bars. (B) Females were less aggressive towards typical males than they are towards purring or silent males. Again, bars not labeled with the same letter differed significantly in female aggression.

song). Typical (ancestral), silent, and purring crickets appear to accrue fitness through survival and reproduction in different ways (Figure 5). Typical crickets are subject to more parasitism (Zuk et al. 2006; Tinghitella et al. 2021), but are more successful than silent and purring males in mate location (Tinghitella et al. 2018a, 2021) and courtship (Tinghitella and Zuk 2009; this paper). Silent crickets avoid parasitism and manage to locate female mates through a combination of alternative mating strategies like increased locomotion and satellite behavior (Zuk et al. 2006; Balenger and Zuk 2015). Despite their inability to produce courtship song at all (which has traditionally been considered a releaser for mounting behavior), around half of females are willing to mate with silent males (Tinghitella and Zuk 2009; this paper). Unparasitized males also have longer adult lives than parasitized males (Simmons and Zuk 1994), which may increase their overall reproductive success. How do the novel purring males accrue fitness, when compared with silent and typical males? Like silent males, purring males are largely protected from parasitism (Tinghitella et al. 2021). They are intermediately successful in long-distance mate location, lagging behind typical males, but outperforming silence (Tinghitella et al. 2018a, 2021). And, finally, purring males appear at least as successful as silent males in courtship contexts (this paper). In summary, each morph seems to have advantages and disadvantages relative to one another in different fitness-related contexts.

We found no evidence of assortative mating (higher mounting rates by females with males from their own population) in this study, although it remains to be seen whether receiver preferences will become coupled with novel signaler traits over time (as described in Broder et al. 2021a). Our current understanding is that

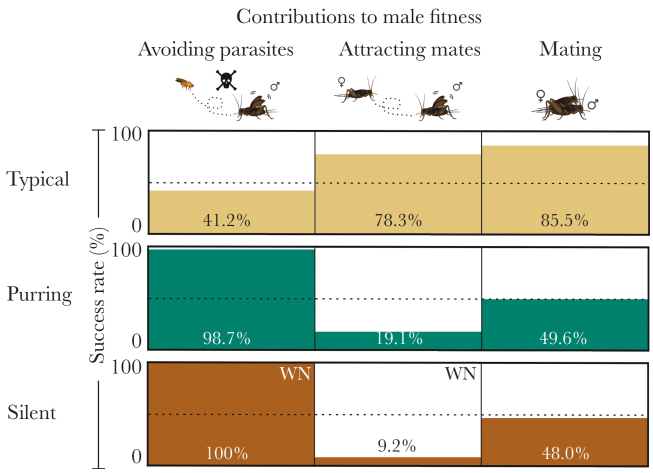

Figure 5
Natural selection and sexual selection pressures shape overall fitness of major morphs of *T. oceanicus* in three key ways during adulthood. Successful males must avoid parasitoids (column 1), locate mates from long-distances (column 2), and convince females to mount during courtship (column 3). Each male morph appears to accumulate fitness differently through survival and reproduction-related behaviors. Here, we place our findings in this paper in the context of related work done in this system previously, to facilitate a broader understanding of how the different morphs accumulate fitness. Column 1 shows that purring and silent males largely escape parasitism, but typical males do not (data from Tinghitella et al. 2021). 98.7% of fly traps broadcasting purring and 100% of traps playing a white noise negative control (WN in the figure) were unattractive to parasitoids, which should lead to very high survival for these male morphs, whereas only 41.2% of traps broadcasting typical noise did not attract parasitoids. Column 2 shows the percent positive phonotactic attraction by female crickets to typical, purring, or white noise tracks played from speakers (data also from Tinghitella et al. 2021). Substantially more female crickets were attracted to typical than purring song, and purring song was more attractive than the white noise negative control (white noise was played to determine whether purring itself is more attractive than just any random sound). Note that the non-zero attraction from afar for silent males (in Column 2) reflects that white noise appears to attract female crickets at a low rate in phonotaxis trials (9.2%; Tinghitella et al. 2021). We suspect that silence is even less attractive than white noise, although silent males also use alternative mate location behaviors like satellite behavior in the field (Zuk et al. 2006). Column 3 presents data from this study and shows the proportion of female crickets across all populations who mounted each type of male in courtship interactions. Again, here we find that purring and silent males are successful in courtship ~50% of the time, whereas typical males are successful in ~85% of trials.

relatively lax mating requirements in Hawaiian populations of *T. oceanicus* (relative to Australian populations, for instance) seem to be a hallmark trait that has facilitated much of the rapid evolution of signaling traits there. In previous work, authors emphasized the importance of relaxed mating requirements and phenotypic plasticity for the initial evolution of silent crickets. Tinghitella and Zuk (2009), for instance, demonstrated that despite courtship song being a releaser for mounting behavior in ancestral Australian populations of the cricket, females from several Hawaiian populations have relaxed that requirement and mate less discriminately overall than females from ancestral populations that do not co-occur with the parasitoid fly. They attribute those relaxed mating requirements to strong population bottlenecks that occurred upon island colonization (Tinghitella et al. 2011) and subsequent selection in small newly founded island populations that favored less discriminating females (Tinghitella and Zuk 2009). Phenotypic plasticity stemming from the acoustic environment (whether individuals hear silence versus song) further relaxes female preferences (Bailey and Zuk 2008) and enhances behaviors that facilitate location of silent males (Zuk et al. 2018). For instance, Bailey and Zuk (2008) found that rearing crickets in silence led to relaxed mating preferences, relative to rearing in typical song, perhaps because the absence of typical song mimics very low mate availability. The animals used in this study were exposed to songs of males from their own population during rearing (mimicking realistic acoustic environments in the wild), so acoustic environment may have contributed to the mating decisions we observed.

Our current data suggest that relaxed mating requirements (relative to elsewhere in the crickets' range) may now be facilitating the origins of novel signals (and signal loss) within Hawaiian populations. In addition to female mating requirements/preferences, what exactly female receivers hear or perceive during the courtship advances of purring and silent flatwing males could explain some of the variation in responses that we see here. We are investigating the role of female sensory systems and vibrational communication (Broder et al. 2021b) in the acceptance of non-typical males in ongoing work. If the relaxed mating decisions we observed here remain over evolutionary time, we predict that multiple morphs of *T. oceanicus* will be maintained within Hawaiian populations, rather than coupled divergence of signals and preferences leading to assortative mating. The behavior of Kalaupapa females in this study is particularly notable; Kalaupapa females, from our predominantly purring population, had higher mounting rates overall than females from Hilo (our representative typical population) or Wailua (the historically silent population). It is curious that Kalaupapa females mounted more readily than did females from Wailua, given the history of 20 + years of predominantly silent crickets in Wailua (Zuk et al. 2018). We suspect that a combination of geographic isolation and very small population size contributes to this pattern. Kalaupapa is an especially small population (often consisting of fewer than 50 adult crickets; personal observation) and occurs in a very remote location on an island where there are no other known populations of this cricket. If the Kalaupapa population experienced a strong bottleneck when it was founded, selection for "eager", relatively non-discriminant females, in combination with the complete lack of typical crickets and thus changed acoustic environment there, may lead to the especially different behavior of Kalaupapa females. In addition to finding interesting patterns in female mounting, we found that females were sometimes aggressive to courting males, and that aggression depended on the males' morph. Females responded to male courtship with aggression more often when the males were a novel morph, rather than the typical, ancestral morph. This pattern could reflect a case of mistaken identity. Compared with typical males, silent flatwing males have feminized cuticular hydrocarbons (CHCs) (Simmons et al. 2014; Pascoal et al. 2020), which are chemical compounds that have a dual purpose of preventing desiccation and identifying the animals' sex (Thomas and Simmons 2009). It is possible that females may mistake silent males for females and thus behave aggressively towards them (Fuentes and Shaw 1986). To our knowledge, the CHC profiles of purring males have not been investigated, but purring males have many morphological characteristics in common with silent flatwing males (Tinghitella et al. 2018a), so it stands to reason that, like silent males, purring males may also have feminized CHC profiles that elicit aggression; this remains untested.

We note two important considerations in this experiment. First, our experimental design largely followed Tinghitella et al. (2009), including the requirement that females undergo two pre-courtship matings prior to focal courtship trials. This method was chosen to minimize effects of virginity on mating decisions, as virgin females sometimes mate indiscriminately (Lickman et al. 1998). We found that females from Hilo were more likely to complete the pre-courtship matings than females from the other populations, which means that the females from Wailua and Kalaupapa used in focal courtship trials may have been more willing to mate with non-typical males than their counterparts who were discarded. If so, the success of the newer male morphs in courtship may be lower (when including all females) than the rates reported here. We hypothesize that Hilo females were more likely to complete pre-courtship matings precisely because they were courted by typical males during these trials (pre-courtship trials were conducted within population only). Second, the patterns we uncovered may be specific to the typical, purring, and silent populations investigated here, and not necessarily to typical, purring, and silent males found elsewhere. Specifically, we chose Wailua, Kalaupapa, and Hilo as study populations for this work because these populations provided the best opportunity to accrue reasonable sample sizes of silent, purring, and typical males (respectively). The morph distributions in Hawaii are in flux and changing rapidly, and in most populations several morphs are found at lower ratios. Here, then, it is possible that morph and population are confounded and it remains to be seen if our results apply more broadly to other populations that contain these morphs across Hawaii.

In this experiment we investigated how one new morph, purring, fares in courtship, relative to the typical and silent morphs that have been most common in this study system for ~20 years. Interestingly, other additional new morphs have recently been described in Hawaiian populations of *T. oceanicus*, including curly-wing, short-wing, and rattling crickets (Rayner et al. 2019; Gallagher et al. in review), suggesting the system is undergoing rapid diversification via a dramatic explosion of phenotypic and genetic variation in song-generating morphology. Anecdotally, we observe changes in the combinations and ratios of different male morphs in different island populations in our biannual trips to the field. As these new morphs are evolving, spreading, and competing with one another, receivers are experiencing changing social (e.g., acoustic and mating) environments likely to influence the fitness of the various morphs. It remains to be seen how each of these additional new morphs fares in the contexts of parasitism, mate location, aggression, and courtship, and how the presence and abundance of the different morphs (sociosexual environments) changes those outcomes. The rapid and increasing evolution of signal novelty in this species provides an outstanding opportunity to understand the myriad selective pressures shaping the earliest stages of sexual signal evolution.

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