

**The costs (and benefits) of standing out: Alternative reproductive behavior and novel trait evolution in the Pacific field cricket**

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## **Dedication**

To the little girl who was never taught to dream.

You are enough.

## **Abstract**

Conspicuous animal signals are often under conflicting selection, with higher performance in one fitness component of life history diminishing performance in another component. The diversity of animal signals in nature suggests that novelty- a new structure or property of an organism that allows it to perform a different function, thus opening a new ‘adaptive zone’ is an important, but often overlooked aspect of signal evolution. The evolution of novelty has largely been absent from conversations about how diversity arises and how it can be maintained. This work seeks to examine both the causes and consequences of novel trait evolution. From individual behaviors to population-level dynamics, I have employed various methodologies for answering a decades-long question in evolutionary biology: how are novel traits accommodated in natural populations?



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## Introduction

How novelty arises has long puzzled evolutionary biologists- particularly how novel animal signals evolve. By nature of their conspicuousness, animal signals are often under conflicting or opposing selection in which higher performance in one fitness component of life history may negatively affect performance in another fitness component of life history. These tradeoffs can constrain the evolution of many traits, especially sexual signals. Yet, the diversity of such signals in nature suggests that novelty has arisen many times over and is an important, but often overlooked aspect of evolution. Animal communication signals are among the most striking features and they often involve a complex mixture of behavior and morphology. However, there are two main problems in studying the influence of behavior on evolution: (1) ancestral behavioral states are difficult to infer; and (2) major evolutionary changes, like the rise and spread of a novel trait, rarely occur on a contemporary timescale.

In the Pacific field crickets, *Teleogryllus oceanicus*, the recent and rapid spread of a novel wing mutation has allowed me to examine the mechanisms, constraints, and evolutionary consequences of novel trait evolution. These crickets are subject to an acoustically orienting parasitoid fly, *Ormia ochracea*, that has been introduced to Hawai'i as well, making calling to attract mates particularly risky. Flexibility in alternative male mating strategies has allowed the persistence and co-occurrence of two male morphs. Because of the costs of calling, non-mutated, *normal-wing* males may either call to attract females, or they may adopt a satellite strategy in which they forego calling, position themselves near callers, and intercept females who are attracted to the

calls. This pre-existing reproductive strategy has allowed for the evolution of a novel male morph, *flatwing*.

Flatwing males have a genetic mutation that greatly reduces the forewing structures necessary for producing sound and are therefore unable to call to attract females. Instead, these males exclusively rely on satellite behavior as a means for attracting mates. While flatwings are protected from parasitism, they face difficulties in securing a mate. Such problems may have been overcome by pre-existing behavioral plasticity, with individuals responding to the increase in silence- due to less calling by normal-wings and the increase in flatwings- by altering their responses to conspecific calling song. In my dissertation, I employed a variety of methodologies to ask questions about how novelty arises, what maintains the presence of a novel trait within a natural population, and what effect do novel traits have on a population.

In chapter 1, I show that novelty arises when subsequent selection on a pre-existing, plastic behavior accommodates rapid changes in the environment. Not all calls are equally favored by selection with females showing a preference for specific calls. Thus, it stands to reason that if males want to optimize their satellite strategy they should settle near calling males with particularly attractive songs. Using empirical, lab-based observations, I show that flatwings, but not normal-wings, prefer calling song models that are most attractive to females. While it is likely that behavioral plasticity in response to the acoustic environment initially allowed for the establishment of flatwing, my results show that differences in reproductive behavior between the two male morphs may have been the result of subsequent selection on flatwings. Despite the disadvantages of obligate silence, flatwings have found a way to increase their reproductive success by

targeting calls that are most likely to attract females (Olzer and Zuk 2018, *Animal Behavior*, 144: 37-43)

In chapter 2, I travel to O‘ahu, Hawai‘i to sample *T. oceanicus* in its natural habitat. Here, I examine the consequences of adaptive plasticity in this signaling system, in which male production of non-preferred signal types could decrease the strength of sexual selection on signals, leading to adaptive signal *degradation*. Over time, plasticity in the expression of mating preferences could contribute to the maintenance of populations by suppressing the expression of female mating preferences when available mates are few, predominantly of non-preferred phenotypes, or both. Spatiotemporal variation in the strength of selection that females impose on signals could also contribute to population divergence by pre-mating isolation. Further, satellite males, specifically flatwings acting as satellites, that no longer express a preference for specific song types could also affect signal evolution over time as males balance the costs, like attracting eavesdropping satellite males or attracting predators, with the benefits, like attracting females. In this chapter, I address a controversial question in the fields of evolutionary biology and animal behavior: the role of plasticity in the adaptive differentiation of wild populations undergoing novel trait evolution. I show that systems under frequency-dependence can hold a preference, but be less choosy depending on the state of the system at the time of sampling. I also show that temporal variation in parasitoid abundance could affect cricket preferences (Olzer et al., *in prep*).

In chapter 3, I show that a stable equilibrium in the proportion of flatwings to normal-wings arises from 2 primary conditions: (1) frequency-dependence in fitness between the two morphs; and (2) at some frequencies, flatwing fitness can exceed

normal-wing fitness. This is important because while flatwing has been relatively successful, it has not been equally successful across all Hawai'ian Islands. Instead, flatwing frequencies vary across 3 Hawai'ian Island populations, suggesting that there are factors influencing the fitness of flatwings in different ways across these populations. As such, we examined what components of fitness are affecting and are affected by the presence of flatwing and how this relates to the rapid rise and spread of the novel mutation. We used an agent-based, spatially explicit simulation modelling environment made available through the Net Logo platform to examine the factors influencing the relative success of flatwing. With this work we have a better understanding of how parasitism and female choosiness can facilitate novel trait evolution in some instances, but hinder novel trait evolution in other instances (Olzer, Rotenberry, and Zuk, *in prep*).

In chapter 4, I look at the other side of the coin from evolution, vestigiality. I show that vestigiality may place constraints on the evolution of novelty as individuals hold on to behaviors that no longer provide the benefit of attracting mates. Here, I examined some of the constraints on the evolution of novelty by looking at stridulation- the act of moving the wings to produce sound- as a vestigial behavior in *T. oceanicus*. While flatwings have lost the wing structures necessary for producing conspecific calling song, and are obligately silent, they continue to express the stereotyped wing motor behaviors- 'stridulating'- produced by non-mutated, normal-wings. I measured stridulation rates in flatwings and normal-wings using substrate microphones. I examined the diel distribution of stridulation in males as a function of morphology and age. I show that differences in the diel distribution of stridulation between the two morphs suggests that flatwings are experiencing relaxed selection on this vestigial behavior. This is

important because this relaxed selection could allow for this vestigial behavior to be co-opted for new purposes in the future. However, relaxed selection could also slow the evolutionary processes, impeding novel trait evolution (Olzer et al. *in prep*).

The work presented here was a collaborative effort. Chapters one and two were co-authored with Marlene Zuk; chapter three with John Rotenberry and Marlene Zuk; and chapter four with Henry Braun, Peter Marchetto, and Marlene Zuk. As such, I retain the use of the first person plural throughout the rest of this dissertation.



**Chapter 1: Obligate, but not facultative, satellite males prefer same male sexual signal characteristics as females**

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## **Abstract**

Signaling is energetically expensive and increases the risk of predation and parasite infection. To balance the costs and benefits of mate attraction, individuals may adopt an alternative mating strategy such as satellite behavior, in which non-signaling males will settle near signaling males and attempt to intercept approaching females. While many suggest that alternative strategies are “making the best of a bad job,” little research has examined whether satellites, particularly obligate satellites, have the potential to increase their mating success by preferentially targeting more attractive signalers. Using the Pacific field cricket, *Teleogryllus oceanicus*, we tested the hypothesis that males are more likely to act as satellites to signaling males with particularly attractive songs, as these callers are likely to attract the most females. In this species, a novel male wing mutation - “flatwing”- renders some males unable to call. Flatwings are obligate satellites, while non-mutated, normal wing males switch between calling and satellite strategies. We presented males with calling song models varying in the percentage of long-to-short chirps, a characteristic important in female choice. As was shown for females in previous work, flatwings exhibited a strong preference for calling songs comprised of 60% long chirp. Normal wing males did not prefer any particular calling song model. Our results lead to the paradoxical conclusion that males with highly attractive songs may not have the highest mating success. Such male preference for male sexual signals may oppose selection by females and increase competition between signaling and satellite males. This could potentially slow the rate of male trait evolution, and could also influence the evolution of male competition.

## **Introduction**

Many animals signal to attract mates (Bradbury and Vehrencamp 2011), but signaling can be energetically expensive (Stoddard and Salazar 2011) and can render individuals vulnerable to increased predation by unintended receivers (Zuk and Kolluru 1998). To ameliorate the costs of attracting mates, individuals may adopt an alternative mating strategy, such as satellite behavior, in which non-signaling males will settle near signaling males and attempt to intercept approaching females (Shuster 2010). This strategy allows individuals to increase their reproductive success, while decreasing the likelihood of encountering predators or parasites (Shuster 2010).

Satellite behavior has been documented in a number of anurans and insects (Gerhardt and Huber 2002; Leary and Harris 2013), birds (Lank et al. 2002; Küpper et al. 2016), and fishes (Tudor and Morris 2009) (Tudor and Morris 2011; reviewed in Neff and Svenson 2015). It remains unclear, however, whether the calling and satellite strategies have equal fitness payoffs. Furthermore, the extent to which satellite males differ amongst themselves with regard to mating success, based on the signaling male they associate with, remains relatively unknown. Because the satellite strategy involves intercepting females that approach signaling males, it stands to reason that if a satellite male is to increase the number of females he mates with, he must increase the number of females he encounters near a signaling male.

But how might satellite males increase the rate at which they encounter females given that this strategy relies on other, signaling, males? To answer this question, Waltz (1982) proposed the Satellite Threshold Model in which he predicted that satellite males should concentrate on areas most attractive to females. Thus, if a male engages in satellite

behavior with a highly attractive calling male, he is likely to encounter and mate with more females than if he acts as a satellite to a less preferred male. However, this hypothesis predicts satellites adopt this alternative tactic based on the attractiveness of the area that a male is occupying (Waltz 1982). In a similar vein, the “relative attractiveness hypothesis” predicts that males adopt a satellite tactic when the attractiveness of a nearby signaling male, exceeds their own attractiveness (Humfeld 2008; Castellano et al. 2009; Berec and Bajgar 2011; Brepson et al. 2012). Often studies of satellite behavior are interested in the rules governing tactic expression in systems where males can switch between various alternative mating strategies throughout their lives. What happens when a tactic is fixed and males must engage in satellite behavior to attract mates?

Further, many of these studies are examining how males group, as in lekking or chorusing species. Instead, we want to understand what happens when individuals show a preference for particular signals that can have a direct impact on their reproductive success. Here we want to address whether an underlying preference exists with the assumption that such a preference has the potential to increase the reproductive success of satellites.

The Pacific field cricket, *Teleogryllus oceanicus*, provides a unique opportunity to test how the “decision rules” regarding satellite behavior are affected by a male’s reliance on this alternative strategy. Like other field crickets, *T. oceanicus* males produce a long-range calling song by rubbing specialized forewing structures together. This song attracts females for mating, but can also attract predators and parasites. In populations of *T. oceanicus* introduced to the Hawai‘ian Islands, calling song attracts an acoustically orienting parasitoid fly, *Ormia ochracea*, that parasitizes and kills calling males (Zuk et

al. 1993; Otte 1994). Male crickets appear to cope with the costs of signaling by engaging in satellite behavior whereby some males will forego calling, settle near a calling male, and intercept approaching females (Zuk et al. 1995).

More recently, in the Hawai‘ian populations of *T. oceanicus*, a novel male morph - “flatwing”- has become established. Flatwing males have a genetic mutation that greatly reduces their forewing structures, rendering them unable to call (Zuk et al. 2006; Tinghitella 2008). Instead, these flatwing males coexist with and capitalize on the calling song produced by non-mutated, normal-wing males as a means of achieving reproductive success (Zuk et al. 2006). While flatwing males must always rely on satellite behavior, normal-wing males engage in a facultative satellite behavior, switching between calling and satellite strategies throughout their lives. Given the risk of calling in these populations, both flatwing and normal-wing males may adopt satellite strategies. However, the importance of this strategy for reproductive success differs depending on whether a male has the option of calling.

Here we test the hypothesis that satellite males may increase their chances of acquiring mates by preferentially settling near more attractive signaling males. We are examining whether males show a preference, the way that females do, for certain signal characteristics, with the assumption that this would allow satellites to access more females and thereby increase their reproductive success. We suggest that if males are capable of discriminating among signaling males, satellite behavior could be favored such that satellites obtain equal or greater fitness to signaling males. For obligate satellites, discriminating among different signal qualities could make the difference between never mating and mating with multiple females. Additionally, if satellites are

shown to discriminate, this can have implications for the success of signalers such that the fitness of a signaling male will depend both on the likelihood of attracting females for mating and the likelihood of attracting rival males. If a male is deemed to have an attractive song by both females and rival males, then his mating success may decrease due to the presence of satellite males near him. Because flatwings are obligate satellites, this study can also help us understand how a drastic change in reproductive morphology (i.e. a change in the underlying wing morphology necessary for sexual signaling) can be accommodated through an alternative strategy. In this case the alternative strategy has become the primary strategy for a subset of males, seemingly with little decrease in reproductive fitness that might be expected if males are, “making the best of a bad job” (West-Eberhard 2003).

To test the hypothesis that satellites of both male morphs discriminate among calling males, we examined how males responded to different calling song models in the lab. Male *T. oceanicus* produce a two-part calling song of long and short chirps (figure 1; Bailey and Zuk 2009). The proportion of long to short chirps has been shown to be particularly important in the context of female mate choice (figure 1; Simmons et al. 2001; Simmons 2004), with females responding fastest to calling song models with 60 percent long chirp (Bailey and Zuk 2008). This female preference is likely influenced by selection on the duration of long chirps by *O. ochracea* (Simmons 2004) where the probability of becoming infested is positively correlated with the duration of long chirps in the calling song (Zuk et al. 1998). Here, we asked: how do males respond to different calling song models? Using playback experiments in the lab, we tested whether males would exhibit a preference, measured in terms of response time, to the 60 percent long

chirp song model (Bailey and Zuk 2008). We predicted that both male morphs would show a preference for the 60 percent long chirp song model, although we expected that flatwings might exhibit a strong preference because they are obligate satellites. We also measured the amount that males moved around in the testing arena. We predicted that males would move around in the testing arena less when they responded positively to the playback speaker, relative to when they responded negatively. Males that hear unattractive songs in the wild will likely move about until they find a male producing an attractive call. Thus, we expect that in the testing arena, males will move around more when they respond negatively to a calling song model.

## **Methods**

### **(a) Study organism**

We used *T. oceanicus* males from the Hawai'ian island of O'ahu. This island has a roughly equal proportion of normal-wing to flatwing males (Pascoal et al. 2014), meaning that males are likely using a satellite strategy to find mates. A *T. oceanicus* laboratory population was originally established using approximately 100 crickets collected from the University of Hawai'i- Manoa campus in 1993 and 1994 (Zuk et al. 1995). Each year the laboratory population is supplemented using offspring of wild-caught females from O'ahu to maintain a minimum of 100 breeding individuals in the colony at all times. We reared crickets in incubators (Caron Insect Growth Chambers model 6025) set at 26°C with 75% humidity on a 12:12 photoreversed light:dark cycle. To avoid any effect of previous mating experience on behavior, we removed males from the large laboratory stocks prior to their final molt. Therefore, sexually-mature males

could not mate with females, ensuring that we only tested virgin males. Males were housed in 15-L containers with about 30 males per container. Inside each 15-L container crickets had access to egg cartons for shelter, and access to water and Fluker's cricket food ad-libitum. We monitored these boxes daily, and then separated each male into an individual, 118-mL cup with a unique identifier upon eclosion. Throughout rearing males were exposed to calling song produced by conspecifics reared in the same chamber. To avoid age-related variation in behavior, we tested males 8-12 days post-eclosion.

### **(b) Song models**

The calling song of *T. oceanicus* males contains 2 important components in female choice: a trill-like long chirp consisting of 6-9 pulses, followed by a series of paired, lower amplitude pulses called short chirps (figure 1.1; Bailey and Zuk 2009). Following Simmons et al. (2001), we constructed song models of varying proportions of long chirp to short chirp using the software package *Raven* v. 1.2 (Ithaca, NY). To maintain the song pulse lengths and the carrier frequency, we excised individual long- and short-chirp pulses from an O'ahu calling song recorded in the wild at 23°C. We then used the excised pulses to construct 4 song models that contained 20%, 40%, 60%, and 80% long chirp, while holding other song parameters constant (see Bailey 2008 for details). Our song models were comparable to average parameters measured in other Hawai'ian *T. oceanicus* populations (Zuk et al. 2001).



### (c) Phonotaxis trials

We assessed male preference by conducting one phonotaxis trial per virgin male in an anechoic chamber at 23-26°C under red light. We performed a total of 153 phonotaxis trials using a 155-cm-long by 34-cm-wide by 31-cm deep arena. On each of the long ends of the arena a speaker behind wire mesh played one of the four song models described above. Males were tested only once to mitigate the effects of prior acoustic experience on phonotaxis behavior that can arise when individuals are repeatedly tested (Wagner et al. 2001). For each trial, we randomly and independently assigned each male one of the four song models. We also randomized the direction of playback for each trial. To approximate the intensity of calling song that males typically experience in the wild, we maintained the playback intensity at 60dB from the release point.

At the beginning of each trial, we positioned a single male 100 cm from the playback speaker and placed a plastic container 11cm in diameter over him. Following a 2-minute silent acclimation period, we began playback of one of the four song models. After 30 seconds of playback we lifted the container and allowed the cricket to freely roam inside the phonotaxis arena. Immediately after releasing a male, we began a 5-minute observation period following the established protocol of Bailey and Zuk (2008). We chose a 5-minute recording period because, observations from the field suggest that if crickets do not begin moving within that period, they are unlikely to move or engage in satellite behavior thereafter (M. Zuk, unpublished observations). Males were undisturbed throughout this 5-minute observation period, during which we measured the following three characteristics of male responsiveness: (i) whether a male moved towards and settled near the playback speaker (within 5 cm of the playback speaker= **positive**

**response**) or moved away from the playback speaker and settled on the side of the arena opposite the playback speaker (**negative response**); (ii) response time: if the response was positive, the latency to settle near the playback speaker; (iii) number of times a male changed directions inside of the testing arena, as a way to assess the strength of his phonotaxis to the playback speaker. Latency to respond to the playback speaker was a proxy for the strength of an individual's phonotaxis behavior and preference, as is used in other studies (Pollack 2003; Bailey and Zuk 2008; Gordon and Uetz 2012; Arbuthnott et al. 2017). Throughout the study, one person observed cricket behavior and one person transcribed the data.

#### **(d) Analyses**

We examined how wing morph affected all three measures of male responsiveness by performing three generalized linear models (GLM) using a maximum likelihood estimation method.

We began by examining how response type (positive- moving toward and settling near the playback speaker vs. negative- moving and settling away from the playback speaker) compared between flatwings and normal wings. We used a GLM with response type as the dependent variable and wing morph and song model as fixed effects (n=154). We also included an interaction term of wing morph by song model. Because the dependent variable had 2 equally likely outcomes (positive or negative response to the playback speaker) we used a GLM with a binomial distribution and a logit link function.

To compare the effect of song model and wing morph on the latency to respond to the playback speaker we used a GLM with response time as the dependent variable and wing morph and song model as fixed effects (n=114). We also included an interaction term of wing morph by song model. Because the data were not normally distributed (Shapiro-Wilk goodness-of-fit;  $p=0.0001$ ), we log-transformed the latency to respond, which normalized the data (Shapiro-Wilk goodness-of-fit;  $p=0.43$ ).

In our last model we examined how wing morph and response type affected the number of direction changes males made before settling either on the same side or on the opposite side of the playback speaker (n=141). We used a GLM with number of direction changes as the dependent variable and wing morph and response type as fixed effects. We also included an interaction term of wing morph by response type. Because our data was heavily weighted toward smaller values for the number of direction changes (supplemental figure 3), we performed a GLM with a Poisson distribution and an identity link function.

Because our three measures of male responsiveness were not affected by pronotum length (i.e. body size; ANOVA:  $p=0.94$ ) or the side of the arena broadcasting calling song during a single trial (ANOVA:  $p=0.82$ ), we did not include these as co-variates in our models. To ensure normality, we used a natural log transformation of response time and number of direction changes for all analyses. We performed all analyses in *JMP* v. 12.2 using a significance level of 0.05.

### **(e) Ethical note**

Our research adhered to ASAB/ABS guidelines for the use of animals in research and the legal requirements under the Animal Welfare Act of the United States Department of Agriculture- Animal and Plant Health Inspection Service (USDA-APHIS permit #P526P-17-03395 to M. Zuk). We collected eggs from gravid females in research sites throughout Hawai'i with permission from USDA-APHIS (permit #P526P-17-03395 to M. Zuk). Laboratory colonies are maintained following all protocol set by USDA-APHIS. Animals were hand collected without the use of animal traps with permission from USDA-APHIS. Laboratory colonies were maintained under conditions that simulated the field environment as closely as possible in temperature- and humidity-controlled incubators. Animals in laboratory incubators were checked daily and given water and food ad-libitum. Animals are housed in 15-L containers with about 30 males per container during rearing. We separated individuals upon eclosion to the penultimate instar, where they were individually housed for up to 12 days post-eclosion. Each individual was tested once 8-12 days post-eclosion and was never reused in the study. At the end of each day, males that were used in phonotaxis trials were euthanized by freezing for at least 24 hours. Males were then autoclaved and disposed of in a biological waste container.

### **Results**

We found no overall effect of wing morph ( $\chi^2_{1, 154} = 0.012$ ;  $p=0.91$ ) or song model on response type ( $\chi^2_{3, 154} = 0.78$ ;  $p=0.86$ ). In about 70% of the trials, flatwings and normal wings responded positively by moving toward the playback speaker (figure 1.2a).

We also found a significant interaction of song model and wing morph on response type such that flatwings responded negatively to the 40% long chirp song model more often than normal wings ( $\chi^2_{3, 154} = 6.62$ ;  $p=0.01$ ; table 1.1). However, overall we found no significant effect of the interaction between song model and wing morph on response type ( $\chi^2_{3, 154} = 7.03$ ;  $p=0.07$ ).

With regard to response time, we found a significant effect of wing morph such that flatwings responded faster than normal wings to all song models ( $\chi^2_{1, 114} = 4.84$ ;  $p=0.03$ ). Flatwings responded an average of 34 seconds faster than normal wings to all song models (Figure 1.2b).

We found a significant effect of song model on response time, with the 60 percent long chirp song model eliciting a significantly faster response ( $\chi^2_{3, 114} = 6.32$ ;  $p=0.01$ ) compared to the reference song model (figure 1.3; table 1.2). We also found a significant wing morph by song model interaction ( $\chi^2_{3, 114} = 4.76$ ;  $p=0.03$ ), indicating that flatwings, but not normal wings, responded significantly faster to the 60 percent long chirp song model (figure 1.3; table 1.2). Overall, flatwings responded 31.6 seconds faster to the 60 percent long chirp model than the 20 percent, 46.9 seconds faster to the 60 percent than the 40 percent, and 74.5 seconds faster to the 60 percent than the 80 percent long chirp song model (figure 1.3).

Lastly, the number of direction changes was affected by both response type ( $\chi^2_{1, 141} = 51.88$ ;  $p<0.0001$ ) and wing morph ( $\chi^2_{1, 141} = 9.46$ ;  $p=0.0021$ ). However, we found no effect of the interaction between wing morph and response type on the number of direction changes ( $\chi^2_{1, 141} = 1.94$ ;  $p=0.16$ ). Overall, males that responded negatively by moving and settling away from the playback speaker, changed direction in the testing

arena significantly more compared with males that responded positively by moving toward and eventually settling near the playback speaker (figure 1.4; table 1.3). In general, flatwings move around the testing arena less than normal wings, regardless of response type (figure 1.4; table 1.3).

## **Discussion**

Several researchers have suggested that behavioral changes within the lifetime of an individual can shift selection such that additive genetic variance is exposed, allowing novel traits to evolve (Duckworth 2009; Hunt 2012). Zuk et al. (2014) argue that more flexible or plastic behaviors should have the greatest influence on the evolution of new traits. While it is likely that behavioral plasticity in response to the acoustic environment initially allowed flatwing to become established in Hawai'i (Tinghitella et al. 2009), this doesn't necessarily explain how flatwing is maintained. Our results suggest that differences in reproductive behavior may have been the result of subsequent selection on flatwings, despite the obvious disadvantages of obligate silence. Even though flatwings and normal wings positively responded to the playback speaker about the same amount, flatwings respond fastest to the 60% long chirp song model, just like females. This suggests that flatwings are not simply engaging in satellite behavior more than normal wings, but instead they are adopting a specialized satellite strategy, in which they preferentially target attractive male signals. In preferentially targeting attractive male signals, flatwings have the potential to encounter more females and increase their reproductive success beyond that of facultative satellites.

Broadly, our work shows that males are discriminating among different signal qualities. Because males did not change direction very much inside the testing arena

when exhibiting a positive response, this suggests that males are following certain decision rules with regard to their satellite strategy. In the wild, males that hear a song that they do not prefer will likely move about until they detect an attractive call, after which they are likely to settle nearby and attempt to intercept approaching females. This is similar to what we are seeing in the testing arena, where males that are responding positively move toward and settle near the playback speaker. However, males that respond negatively will change directions and move around more before settling as far away from the playback speaker as is possible in the testing arena. While we know that males will sometimes associate with other males that females deem particularly attractive, especially in chorusing and lekking species (Beehler and Foster 1988; Pfennig et al. 2000), our study is the first to demonstrate this phenomenon in a system with an obligate satellite strategy, where adopting a “good” satellite strategy (i.e. one that increases the chances of encountering females) is especially important because males have no other means of attracting mates.

Such discrimination raises a larger question about how male attendance to male signals affects the evolution of secondary sexual characteristics. Much research shows that female preference for male signals can drive the evolution of elaborate male traits (reviewed in (Rosenthal 2017), little parallel research has examined the evolutionary causes and consequences of male attendance to male sexual signals- though we have some evidence that males may assess their opponents in agonistic contests (Stuart-Fox 2006; Painting and Holwell 2014). Here we arrive at a paradoxical conclusion in which males with exceptionally attractive songs do not always have the highest mating success. Even though females prefer calling songs with 60 percent long chirp, males producing

such songs are also more likely to attract satellite males. Therefore, male preference for male signals may actually oppose selection by females on male sexual traits, potentially slowing the rate of male trait evolution. At the same time, this could increase competition between calling and satellite males, further driving selection on mate attraction. Our work suggests an area of future research on sexual selection via male-male preference.

The ability to discriminate among signaling males may be a window into how satellite behavior is maintained. Much research has been devoted to the question of how discontinuous variation in male mating behavior is maintained (reviewed in Oliveira et al. 2008), and while many suggest that alternative strategies are “making the best of a bad job” (West-Eberhard 2003), this need not be the case. Previous work has shown that satellite behavior can be as successful as calling in some cricket populations (Rowell and Cade 1993; Walker and Cade 2003). Here we show that by discriminating among callers, satellite males may gain greater access to females while also escaping the costs associated with signaling. This may provide equal or even greater fitness to satellites. Because of the potential for equal fitness benefits and functional equivalence, many have concluded that like evolutionarily stable strategies, alternative tactics are maintained by frequency-dependent selection particularly with regard to conditional versus mixed strategies (Plaistow et al. 2004). Further work will explore the role of frequency-dependence in satellite behavior and the success of flatwing.

### **Author contributions**

The experiment was designed by R.M.O and M.Z. The data were collected by R.M.O. The data analysis was carried out by R.M.O. The manuscript was prepared by R.M.O. and was critically reviewed by M.Z.



### **Competing interests**

The authors do not have any competing interests in this research.

### **Data Availability**

The raw data for this study can be found in the Mendeley Data repository (<https://doi.org/10.17632/sbr49bbysb.1#file-e1579123-58ab-43bb-a6d8-6779e1388024>)

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## Tables

Term	Estimate	Std Error	X squared	p-value
Intercept	-1.26	0.27	27.22	<0.0001*
Wing Morph [FW]	-1.23	0.27	0.012	0.91
Song model (20% long chirp)	-1.65	0.49	0.72	0.4
Song model (40% long chirp)	-1.17	0.45	0.039	0.84
Song model (60% long chirp)	-1.06	0.42	0.22	0.64
Wing Morph [FW] * Song model (20% long chirp)	-1.83	0.49	1.6	0.21
Wing Morph [FW] * Song model (40% long chirp)	0.12	0.45	6.62	0.01*
Wing Morph [FW] * Song model (60% long chirp)	-1.18	0.42	0.03	0.86

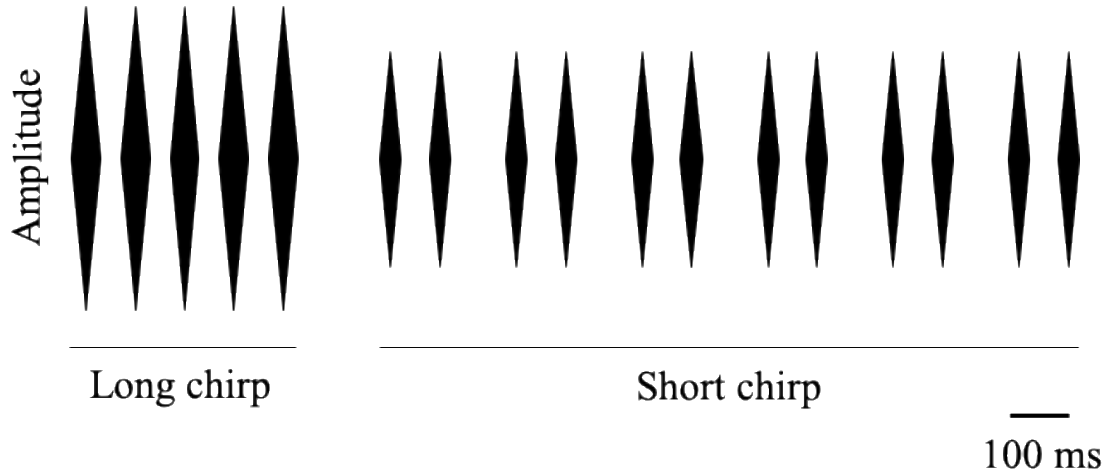
**Table 1.1** Summary table of Generalized Linear Model with response type (positive versus negative) as the dependent variable and wing morph, song model, and an interaction of wing morph by song model as the fixed effects (n=154).

<b>Term</b>	Estimate	Std Error	X squared	p-value
Intercept	4.31	0.075	386.09	<0.0001*
Wing Morph [FW]	4.14	0.075	4.84	0.03*
Song model (20% long chirp)	4.32	0.11	0.022	0.88
Song model (40% long chirp)	4.38	0.15	0.28	0.62
Song model (60% long chirp)	3.98	0.13	6.32	0.01*
Wing Morph [FW] * Song model (20% long chirp)	4.25	0.11	0.21	0.65
Wing Morph [FW] * Song model (40% long chirp)	4.44	0.15	0.85	0.35
Wing Morph [FW] * Song model (60% long chirp)	4.02	0.13	4.76	0.03*

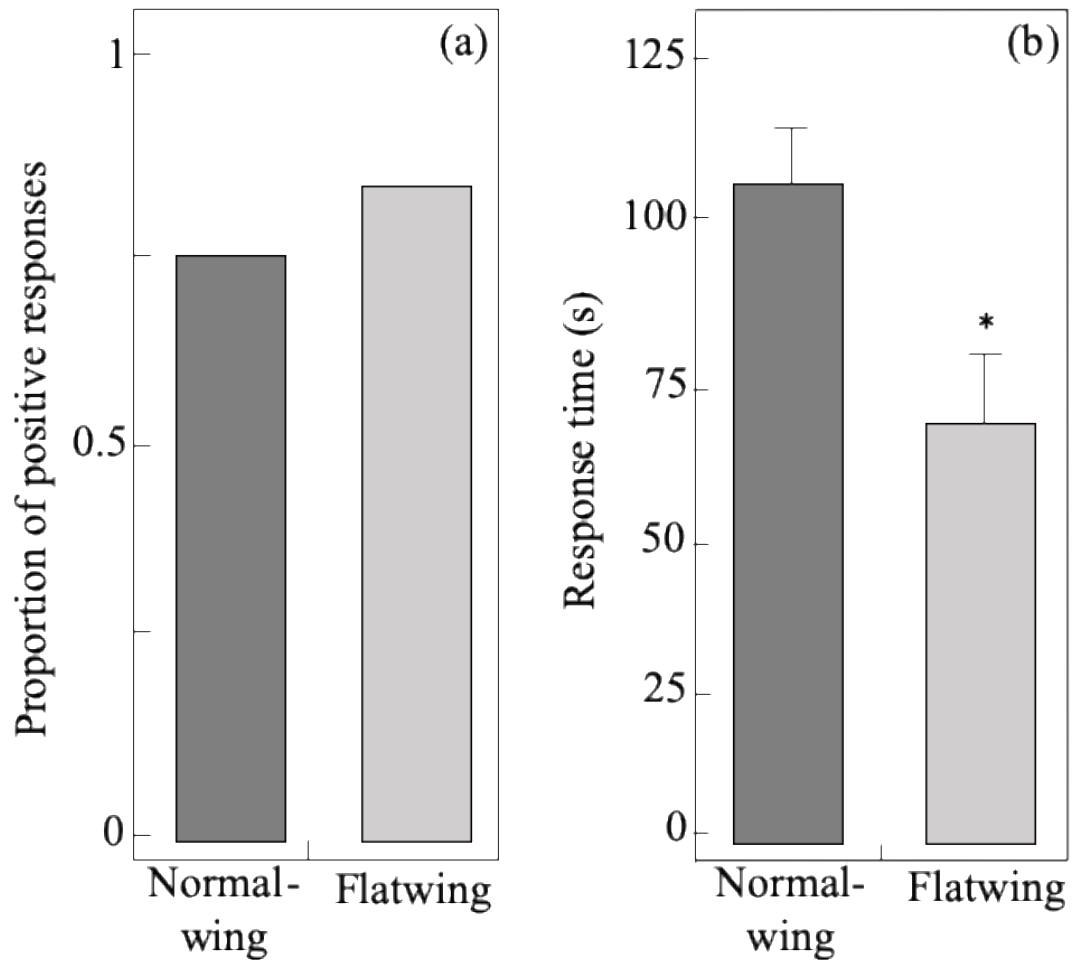
**Table 1.2** Summary table of Generalized Linear Model with response time as the dependent variable and wing morph, song model, and an interaction of wing morph by song model as the fixed effects (n=114).

<b>Term</b>	Estimate	Std Error	X squared	p-value
Intercept	2.53	0.2	160.36	<0.0001*
Wing Morph [FW]	1.84	0.2	9.46	0.0021*
Response [Neg]	3.69	0.2	51.88	<0.0001*
Response [Neg]* Wing Morph [FW]	2.24	0.2	1.94	0.16

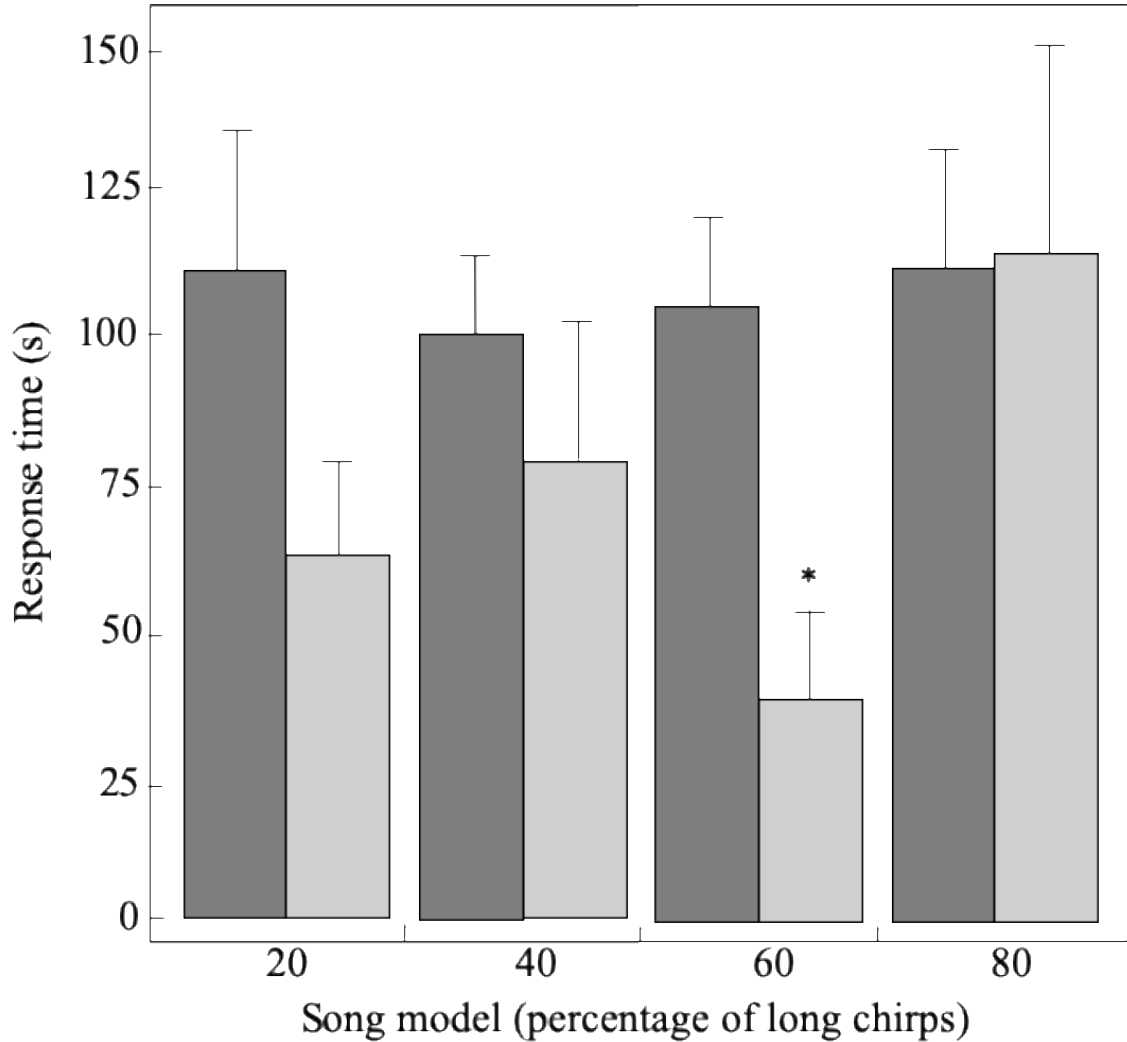
**Table 1.3** Summary table of Generalized Linear Model with the number of direction changes toward and away from the playback speaker as the dependent variable and wing morph, response type (positive versus negative), and an interaction of wing morph by response type as the fixed effects (n=141).



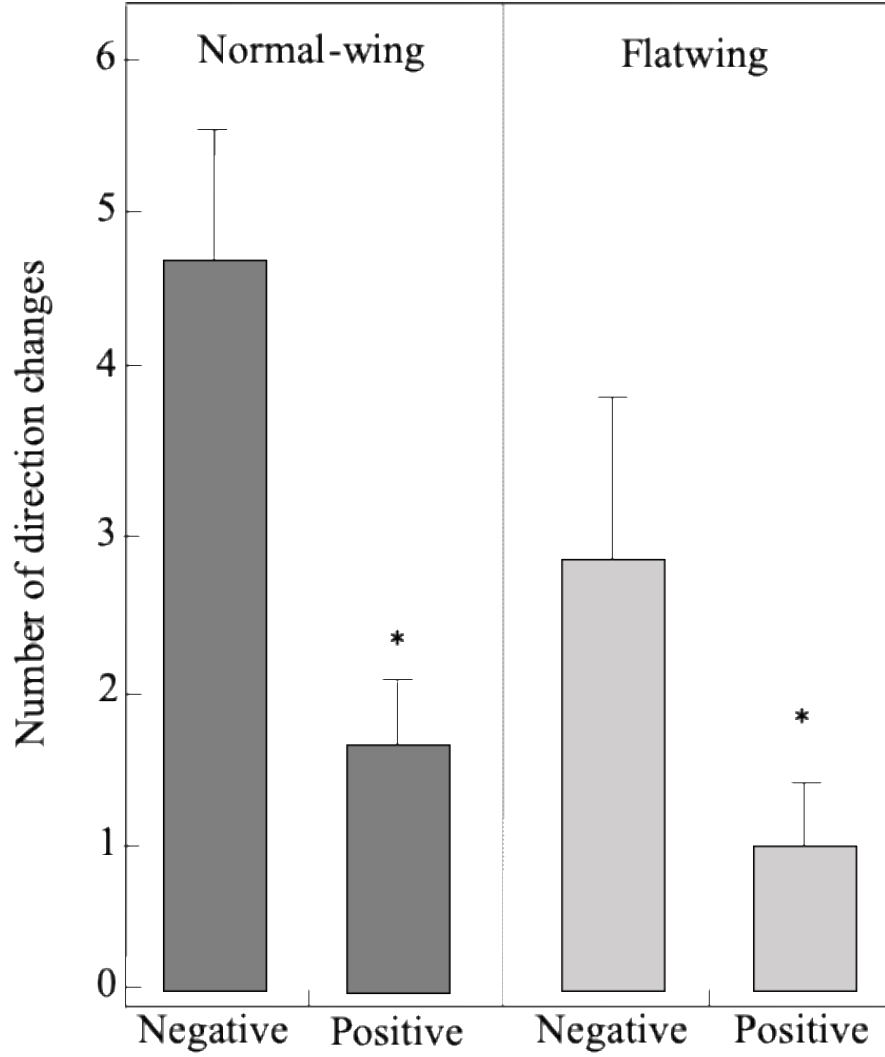
**Figure 1.1** Oscillogram of *T. oceanicus* calling song from Bailey and Zuk 2009. Each of the single black lines represents a pulse in the cricket song. Males produce a long-range song by rubbing specialized wing structures together. The song consists of 2 discrete phrases, the long chirp which is comprised of 3-8 sound pulses, followed by a series of short chirps each with 2 sound pulses



**Figure 1.2** (a) Proportion of males positively responding by moving toward and settling near the playback speaker (N=154). Normal wings and flatwings contacted the speaker approximately the same amount. (b) Latency of males to positively respond to the playback speaker (N=118). Bars represent 1 SE. We tested each male once and during a 5-minute observation period, recorded (1) whether a male responded positively to the playback speaker and (2) if he responded positively, how long it took to settle near the playback speaker. Flatwings responded 34.32 seconds faster to the playback speaker than normal wings ( $p=0.03$ ).



**Figure 1.3** Latency of males to settle near the playback speaker broadcasting each of the song models (n=114). Normal wings are represented in dark grey; flatwings are in light grey. Bars represent 1 SE. We tested each male once and measured whether or not he responded positively by moving toward and settling near the playback speaker. If a male responded positively, we also measured his response time as the latency to settle near the playback speaker. We found a significant interaction effect of song model by wing morph on response time ( $p=0.03$ ). Flatwings responded significantly faster to the 60 percent long chirp song model, just as females were previously found to do.



**Figure 1.4** Number of direction changes when a male responded either positively by moving towards and settling near the playback speaker, or negatively by moving and settling away from the playback speaker (N=141). Error bars represent 1 SE. We tested each male once. During a 5 min observation period, we measured the number of times a male changed direction in the testing arena relative to the playback speaker. ( $p < 0.01^*$ ).



**Chapter 2: Field crickets do not express calling song preference in presence of a parasitoid**

In review at:  
The Canadian Journal of Zoology

## Abstract

Many mating behaviors are context-dependent and can be strongly influenced by the ecological and social environment. Here, we examined the reproductive behaviors of the Pacific field cricket, *Teleogryllus oceanicus* by measuring male and female preferences for certain male song characteristics using playback speakers in the field. Unlike previous studies performed in a laboratory setting, we found no strong evidence that males or females are exercising a preference for certain male calling song characteristics in the wild. We also measured the abundance of an acoustically orienting parasitoid of *T. oceanicus* and compared this with five years of previous parasitoid abundance data. These comparisons showed that parasitoid abundance during the year of this study was higher than any of the previously recorded abundances. Our results suggest that wild crickets are responding to their social environment in a plastic manner. We hypothesize that high densities of the parasitoid drove mate availability down in our study, and that this resulted in the suppression of preferences for attractive male calls.

## Introduction

Many mating behaviors, like female preferences and male signaling, are context-dependent (Cornwallis and Uller 2010; Milner et al. 2010; Prudic et al. 2011; Gillespie et al. 2014; Miller and Svensson 2014). As a result, environmental heterogeneity, particularly across space and time, can cause fluctuations in sexual selection by altering the dynamics of female mate choice and male competition (Siepielski et al. 2009; Siepielski et al. 2011; Siepielski et al. 2013; Miller and Svensson 2014). Both predation risk and the availability of mates can affect both signaling and choosiness behavior. While controlled, laboratory settings are a common setting within which to study reproductive behavior, these environments often lack the environmental heterogeneity necessary to elicit variation in mating behaviors. Most laboratory settings are devoid of predators and parasites, are kept at a constant temperature, and individuals often have access to ample resources, including an abundance of mating opportunities. Because animals living and breeding in captivity experience markedly different social environments from their wild counterparts (Frankham 2008; Christie et al. 2016; Olzer et al. 2019), being able to see how behavior under controlled conditions differs from that exhibited under more natural conditions, like in the field, can allow us to better understand the mechanisms driving variation in mating behavior.

We have an opportunity to compare reproductive behavior in the field and in the lab using the Pacific field cricket, *Teleogryllus oceanicus*. Male *T. oceanicus* signal to attract females by rubbing specialized structures on their forewings together to produce calling and courtship songs, as such calling song is subject to sexual selection by females who tend to prefer certain song types over others. While *T. oceanicus* is native to

Australia and islands throughout the South Pacific, in populations introduced to the Hawai‘ian Islands, calling song is also subject to selection by an acoustically orienting parasitoid fly, *Ormia ochracea*, also introduced to Hawai‘i (Otte 1994; Zuk et al. 1993). Female *O. ochracea* locate a calling male, deposits free-moving larvae around them that then burrow inside a male’s body, develops within him, and emerges as an adult- killing the male in this process (Cade 1975; Zuk et al. 1993). In Hawai‘i, there has also been the recent spread of a novel male type, *flatwing*. Flatwings have greatly reduced forewing structures necessary for producing conspecific calling song, and are instead obligately silent (Zuk et al. 2006). Flatwings appear to circumvent the problem of mate attraction to song by relying on a common alternative mating strategy, satellite behavior, in which males will forego signaling, settle near a signaling male, and attempt to intercept approaching females (Tinghitella et al. 2009). Males without the new mutation still call, but will also facultatively engage in satellite behavior (Zuk et al. 1995; Tinghitella et al. 2009). Flatwings comprise about 50% of the males on the island of O‘ahu, 90% of the males on Kaua‘i, and about 2% on the big island of Hawai‘i (Zuk et al. 2018).

In laboratory populations of *T. oceanicus*, both males and females have shown a strong preference for certain calling song characteristics. Male *T. oceanicus* produce a two-part calling song that consists of long and short chirps (Bailey and Zuk 2009). The proportion of long-to-short chirps in the calling song is particularly important in the context of female choice as well as male satellite behavior. Female *T. oceanicus*, as well as flatwing males acting as satellites, preferentially respond, by moving and settling fastest, to calling song models that contain 60% long-chirp (Bailey and Zuk 2008; Olzer and Zuk 2018). This female preference is likely influenced by selection by *O. ochracea*

where the probability of becoming infested is positively correlated with the duration of long chirp in the calling song (Zuk et al. 1998; Simmons 2004). Flatwing male preference has likely evolved in response to the likelihood of encountering females when relying on a satellite strategy as the sole means of obtaining mates (Olzer and Zuk 2018). However, laboratory-reared *T. oceanicus* exhibit a high degree of plasticity in their reproductive behaviors. Males and females are highly sensitive to cues from their social environment during development and will respond by altering their mating behavior in adulthood. Females reared in a laboratory without calling song, mimicking an environment with few calling males, are less choosy in adulthood. (Bailey and Zuk 2008). This may be because females use the amount of calling song as a cue for the number of potential mates available in the environment.

Male *T. oceanicus* are also sensitive to social cues and when reared in a call-less, laboratory environment are more likely to engage in satellite behavior in adulthood (Bailey et al. 2010). This type of plasticity in response to the social environment may enable the flatwings to persist in the face of rapid environmental change, like changes in parasitoid pressure. Changes in parasitoid abundance can in turn, affect the abundance of calling males from which females can choose. This would be particularly true in the wild where conditions like predation not only differ from the laboratory but are often temporally and spatially variable. What remains unknown, however, is the extent to which *T. oceanicus* in the wild shows these same mate or satellite preferences. While we have monitored female and male abundances, as well as predator abundances since the discovery of flatwing in 2005, we have yet to measure preferences for calling song characteristics outside of a controlled, laboratory setting.

Here, we examine whether female preferences, as measured by phonotaxis behavior (i.e. attraction to sound) and male satellite behavior are expressed under more natural conditions where important ecological and social factors are markedly different—notably, the presence of the parasitoid fly. Examining mating behavior in the wild allows us to determine how the presence and abundance of the parasitoid fly might influence short-term plasticity in phonotaxis. For instance, parasitism may change how reliant wild males are on satellite behavior. If males are more reliant on satellite behavior for finding mates, then they might be more likely to exhibit a preference, so that the costs of acting as a satellite to an unattractive male are minimized. Further, if fly abundances are driving normal-wing populations down, crickets experience less exposure to song during development. If those wild crickets are developing in a song-less environment, they may respond similarly to how laboratory-reared crickets have: adult females may be less choosy, and adult males may be more likely to engage in satellite behavior. This would allow the population to persist in the presence of predation, as females mate less discriminately with males.

We examined male and female preferences by testing attraction to various calling song models in a natural setting. We conducted playback experiments in the field in which we randomly selected one of four calling song models that varied in percent long-chirp to broadcast for 20-min, and measured two metrics of preference: (1) the number of crickets that responded to the playback speaker and (2) the distance that each cricket settled from the playback speaker. We were also interested in whether parasitoid fly abundances were associated with differences in the abundance of males and female crickets. We measured parasitoid abundance as the number of flies resting on the speaker

after 20 minutes of playback and compared the abundance from 2019 with 5 years of *O. ochracea* abundances from 2010-2013 and 2015. We predicted that in the wild, as in the lab, females would show a preference for 60% long chirp song model. Similarly, we predicted that if females exhibited a preference for specific calling song models, flatwings would also exhibit this preference, because the benefits of acting as a satellite to certain males is only present if females are more likely to move towards these males as well. If females and males have a preference for a particular song model, we expect there to be more crickets as the speakers broadcasting this song model, and for the crickets to settle closer to these speakers.

We predicted that if females did not show a preference for any song model, then flatwings would also show no preference. We predicted that females and flatwings would only show a preference if the risk of parasitism was relatively low and there were equal or more normal-wings than flatwings at the playback speaker. We had no prior assumptions about normal-wing behavior.

## **Methods**

### **(a) Field site**

We performed playback experiments at two lawns located west and east of Academic Circle on the Brigham Young University-Hawai'i campus in La'ie on the west side of the island of O'ahu in Hawai'i. To the west of Academic Circle, lawn A measures roughly 25,000 sq. meters. To the east of Academic Circle, lawn B measures roughly 40,000 sq. meters.

Data were collected on 11 nights during June 2019. Each night we randomly picked one of the two lawns to survey. We performed 6-8 phonotaxis trials each night beginning 1 hour after sunset (approx. 8pm HST). Data collection finished c. 9:30 pm every night. We recorded ambient temperature and humidity at the onset of the first playback and at the conclusion of the last playback of the night. Temperatures ranged from 21.1°C to 27.9 °C with humidity ranging from 60-83%. We immediately halted data collection if it began to rain during the night, and discarded any data collected during rainfall.

### **(b) Song models**

The *T. oceanicus* calling song contains two important components: a trill-like long-chirp composed of 6-9 pulses, followed by a series of short chirps that are paired, lower-amplitude pulses. The proportion of long-to-short chirps in the calling song is important in mate choice (Bailey and Zuk 2009) and for the male satellite strategy (Olzer and Zuk 2018). In laboratory experiments, females preferentially responded to calling song models with 60% long chirp (Bailey and Zuk 2008). In follow-up studies with laboratory-reared males, flatwings preferentially respond to calling song models with 60% long-chirp; however, normal-wings did not exhibit any preference for a specific calling song model (Olzer and Zuk 2018). Following Simmons et al. 2001 and using the software package Raven v1.2 (Cornell Lab of Ornithology), we constructed four song models of varying proportions of long-to-short chirps: 20%, 40%, 60%, and 80%. We used excised pulses from an O‘ahu calling song recorded in the wild at 23°C. All details of how song models were constructed can be found in Olzer and Zuk (2018). Our song



models were comparable to average parameters measured in other Hawai‘ian *T. oceanicus* populations (Zuk et al. 2001).

### **(c) Phonotaxis trials**

To measure phonotactic responses and preference, we performed playback experiments in the field following the protocol of Zuk et al. (2018). After delineating a 2-m radius circle in one of the two lawns on the BYU-Hawai‘i campus, we collected any crickets inside and recorded the sex, wing morph, and number of each of the 3 types of crickets (female, normal-wing, flatwing) before removing them from the circle.

We then placed a speaker in the center of the circle and randomly selected one of the 4 calling song models to broadcast for a 20-minute playback period. We calibrated the speaker to a sound-pressure level of 70 dB SPL at 1 meter from the center of the circle. At the end of the 20-minute playback period, we recorded the number of flies resting on the speaker before turning off the speaker. We then collected any crickets that had come into the circle and noted the sex, wing morph (if male) and distance from the speaker that each cricket was found. To minimize observer bias, blinded methods were used when all behavioral data were recorded and analyzed.

### **(d) Comparison of parasitoid fly abundance across years**

We measured abundance of the parasitoid fly, *O. ochracea*, during the 2019 field season as the number of flies resting on the playback speaker after 20 minutes of playback. We compared these data with previous collection years at the same site in

which flies were lured to a playback speaker using a normalized calling song model (see Zuk et al. 2018 for more details).

## **Analyses**

We examined the effect of sex, wing morph, and song model on two measures of preference: the number of crickets at each speaker and the distance settled from the playback speaker. We did this by performing a series of Generalized Linear Mixed Models (GLMMs).

We began by comparing the number of crickets of each sex (males vs. females) at the playback speakers. We used a GLMM with number of crickets as the response variable, sex and song model as fixed effects, and location (lawn A vs. lawn B) and night as random effects. We also included an interaction term of sex by song model as a fixed effect.

We also compared the number of males of each morph (flatwings and normal-wings) at the playback speaker using a GLMM with number of males as the response variable, wing-morph and song model as fixed effects, and location (lawn A vs. lawn B) and night as random effects. We also included an interaction term of wing morph by song model as a fixed effect.

To examine the effect of sex and song model on the distance settled from the playback speaker, we used a GLMM with log distance settled as our response variable, sex, song model, and a sex by song model interaction as fixed effects, and night as a random effect.

We also examined the effect of wing morph and song model on distance settled. We ran a GLMM with log distance settled as the response variable, wing morph, song model, and a wing morph by song model interaction as fixed effects, and location as a fixed effect. Again, we used a log transformation of distance settled to normalize the data.

We tested the effect of song model and sample year on the number of parasitoid flies present on the playback speaker. First, we performed a GLMM with number of flies as the response variable, song model as the fixed effect, and night as a random effect. To examine the effect of sample year, we performed a Generalized Linear Model (GLM) with the number of flies as the response variable and year as the fixed effect. Because the number of flies follows a negative binomial distribution (Pearson  $\chi^2=194.16$ ;  $p=0.31$ ), we used a GLM with a binomial distribution, log link function, and maximum likelihood estimation method.

Because temperature and humidity can vary greatly between laboratory and natural environments, we examined the effect of average temperature and average humidity on all response variables. To examine the effect on distance settled from the playback speaker, we used a GLM with log-distance settled as the response variable, a normal distribution, log link function, and maximum likelihood estimation method (N=429). To examine the effect on the number of crickets at the playback speaker, we used a GLM with the number of crickets as the response, a binomial distribution, log link function, and maximum likelihood estimation method (N=66). To examine the effect of temperature and humidity on the number of flies at the playback speaker, we performed 2

GLMs with number of flies as the response variable, a binomial distribution, and log link function (N=17).

## Results

Significantly more females than males (figure 2.1; GLMM:  $F_{1, 153.6}=68.27$ ;  $p<0.0001$ ) and more flatwings than normal-wings were collected at the playback speaker (figure 2.1; GLMM:  $F_{1, 97.7}=19.93$ ;  $p<0.0001$ ).

Song model (percent long-chirp) did not affect the number of crickets at the playback speaker (figure 2.2; GLMM:  $F_{3, 156.7}=1.02$ ;  $p=0.38$ ), and there was no interaction between song model and sex (GLMM:  $F_{3, 153.6}=1.25$ ;  $p=0.29$ ) or song model and wing morph (GLMM:  $F_{1, 97.7}=0.42$ ;  $p=0.74$ ).

Females settled closer than males to the playback speaker (figure 3; GLMM:  $F_{1,478.9}=14.63$ ;  $p<0.0001$ ). Flatwings and normal-wings settle the same distance from the playback speaker (figure 2.3; GLMM:  $F_{1,164.9}=0.33$ ;  $p=0.57$ ). Based on two separate Generalized Linear Models (GLMs) night and location did not have an overall effect on distance settled (GLM, night:  $\chi^2_{10} = 11.91$ ;  $p=0.29$ ; GLM, location:  $\chi^2_1 = 1.29$ ;  $p=0.26$ ). However, one night had a significant effect on distance settled ( $\chi^2 = 4.76$ ;  $p=0.03$ ), so we included night in our model as a random effect. Because distance settled did not initially follow a normal distribution (Shapiro-Wilk W Goodness-of-fit test:  $W=0.96$ ;  $p<0.0001$ ), we did a log transformation that normalized the data.

All crickets settled the same distance regardless of song model presented (figure 2.3; GLMM:  $F_{3, 177.0}=2.00$ ;  $p=0.12$ ). Further, there was no significant interaction between

sex and song model (GLMM:  $F_{3, 478.3}=0.77$ ;  $p=0.51$ ) or wing morph and song model (GLMM:  $F_{1, 188.3}=0.42$ ;  $p=0.74$ ).

Song model did not affect the number of flies at the playback speaker either (GLMM:  $F_{3, 27.4}=0.22$ ;  $p=0.88$ ). However, sample year had a significant effect on the number of flies, such that 2019 had significantly more flies than any previous year (figure 2.4; GLM:  $\chi^2_5= 18.95$ ;  $p=0.002$ ).

Lastly, temperature and humidity had no significant effect on the number of crickets (avg. temp, GLM:  $\chi^2_1= 0.01$ ;  $p=0.90$ ; avg. humidity, GLM:  $\chi^2_1= 0.11$ ;  $p=0.75$ ) or the distance that crickets settled from the playback speaker (avg. temp, GLM:  $\chi^2_1= 1.61$ ;  $p=0.20$ ; avg. humidity, GLM:  $\chi^2_1= 0.42$ ;  $p=0.52$ ). Temperature had no significant effect on the number of flies at the playback speaker (ANOVA:  $\chi^2_1= 2.92$ ;  $p=0.09$ ) and neither did humidity (ANOVA:  $\chi^2_1= 0.60$ ;  $p=0.44$ ).

## Discussion

Unlike crickets in the laboratory, *T. oceanicus* in the wild are not exercise a strong preference for any particular calling song models. In laboratory studies of *T. oceanicus*, females preferentially responded to calling song models that contained 60% long-chirp (Bailey and Zuk 2008). Similarly, laboratory-reared flatwings preferentially responded to the same calling song models that females preferred (Olzer and Zuk 2018). Unlike laboratory crickets, wild female *T. oceanicus* settled the same distance from all calling song models and were found in the same abundance at all speakers regardless of song model presented. This suggests that females may not be discriminating between different male signal qualities when choosing a mate in the wild.

This different responses of wild and laboratory crickets may be the result of adaptive phenotypic plasticity in which crickets are responding to their immediate environments, rather than responding the same way regardless of what they experienced or heard during rearing. In laboratory studies, *T. oceanicus* plastically respond to alterations in their social environment. Females reared in an environment devoid of male calling song are less choosy as adults (Bailey and Zuk 2008);. Conversely, females reared with song preferentially respond to the 60% long-chirp song model in the laboratory (Bailey and Zuk 2008). It is likely that female *T. oceanicus* evaluate the amount of calling song in their environment as a proxy for the number of potential mates. Broadly, as mate availability decreases and populations become more female-biased, female choosiness is expected to decrease (Emlen and Oring 1977). In this case, females may be attempting to decrease the costs associated with rejecting a male when few are available to mate with. Furthermore, when mate availability decreases, females may spend more time searching for males before encountering a potential mate (Ferreira et al. 2018). Decreased encounters with potential mates can also affect how females assess phenotypic variation in the population (Gowaty and Hubbell 2009; Ah-King and Gowaty 2016). All of this increases the costs associated with being choosy and indeed theoretical models of sexual selection state that a reduction in choosiness can be adaptive when mate availability is limited (Kokko and Mappes 2005). What works for animals under laboratory conditions, may not be adaptive in the field.

One particularly important way that the social environment may be altered is through predation. Predation can directly affect the number of males, and thus, potential mates from which females can choose. While there are many differences between field

and laboratory environments, such as temperature and humidity, we found no effect of these ecological factors on cricket behavior in the wild. However, other factors related to microhabitat, such as light availability, can effect behavior and cause differences between wild and laboratory populations (Owens and Lewis 2018; Palomar-Abesamis et al. 2018).

Differences in behavior between the laboratory and field crickets may be explained by the presence or absence of a parasitoid. By nature of their conspicuousness, sexual signals often subject signalers, typically males, to increased predation (Zuk and Kolluru 1998). Increased predation may thus decrease the number of signaling males from which females can search, sample, and choose (Edomwande and Barbosa 2020). In our system, as predation risk increases, normal-wing frequency presumably decreases, resulting in more silence in the environment. This can result in decreased female choosiness. In this study, we found more parasitoids than in with the previous 5 years sampled. Additionally, *T. oceanicus* populations on O‘ahu typically have a 50:50 flatwing to normal-wing ratio (Zuk et al. 2006; Zuk et al. 2018). However, during this study we recorded significantly more flatwings than normal-wings at all playback speakers. This suggests that predator abundances may be driving normal-wing populations down, leading to increased silence and a decrease in female choosiness.

Like females, males did not exhibit a preference for any calling song models in the wild. This result was in line with our prediction that if females did not exhibit a preference in the wild, than males would not exhibit a preference either. While flatwing males in the laboratory preferentially responded to calling song models with 60% long chirp, we expect this preference to be contingent on a benefit that being choosy will lead to increased encounters with females. In other words, if females prefer males that have

calling songs containing 60% long chirp, then they should preferentially move towards and attempt to mate with these males. Thus, satellite males would benefit from settling near more attractive males, as this would increase the likelihood of encountering and mating with females while employing this alternative reproductive tactic. In the field there may not be a benefit to acting as a satellite to some males over others, and indeed we saw no strong preferences exhibited by either male morphotype.

Though our study revealed that males and females are not exercising a strong preference in the field, we found some interesting broadscale patterns in phonotaxis behavior. Most notably, flatwings appear more phonotactic than normal-wings. In laboratory studies, males of each phenotype reared without conspecific calling song are more likely to engage in satellite behavior during adulthood (Bailey et al. 2010). This could mean that while males are not being choosy in the field, they are actually more likely to engage in satellite behavior when there are fewer callers in the environment. With the lack of female choosiness, this may actually benefit satellite males, as females may be more likely to mate with them upon encounter. This could be particularly beneficial for flatwings who rely exclusively on satellite behavior as a means for achieving reproductive success. Alternatively, flatwing males may be exhibiting different behaviors due to differences in mating histories that could be the result of not producing a calling or courtship signal. While differences in female encounter rates between flatwings and normal-wings have not been demonstrated in the field, theoretical models suggest that differences in encounter rates is a contributor to differences in mating success between the morphs (Rotenberry and Zuk 2016). Conversely, differences



in behavior could be the result of differences in genetics or pleiotropic effects of the flatwing genotype (Pascoal et al. 2014; Heinen-Kay et al. 2019).

Differences between laboratory and wild cricket behavior could also be the result of evolutionary divergence between crickets living and breeding in markedly different environments. Because animals living and breeding in captivity often experience dramatically different conditions than their wild counterparts, this can lead to evolutionary divergence between captive and wild populations (Frankham 2008; Christie et al. 2016). In field crickets, *Gryllus campestris*, behavior in captivity predicts some aspects of wild behavior, but not all (Fisher et al. 2015). Individual behaviors like activity and exploration were similar between laboratory and field animals (Fisher et al. 2015). However, traits in the wild were predictably influenced by environmental factors such as temperature and sunlight (Fisher et al. 2015). These results highlight the importance of validating the results of laboratory studies by comparing similar behaviors in the wild (Fisher et al. 2015).

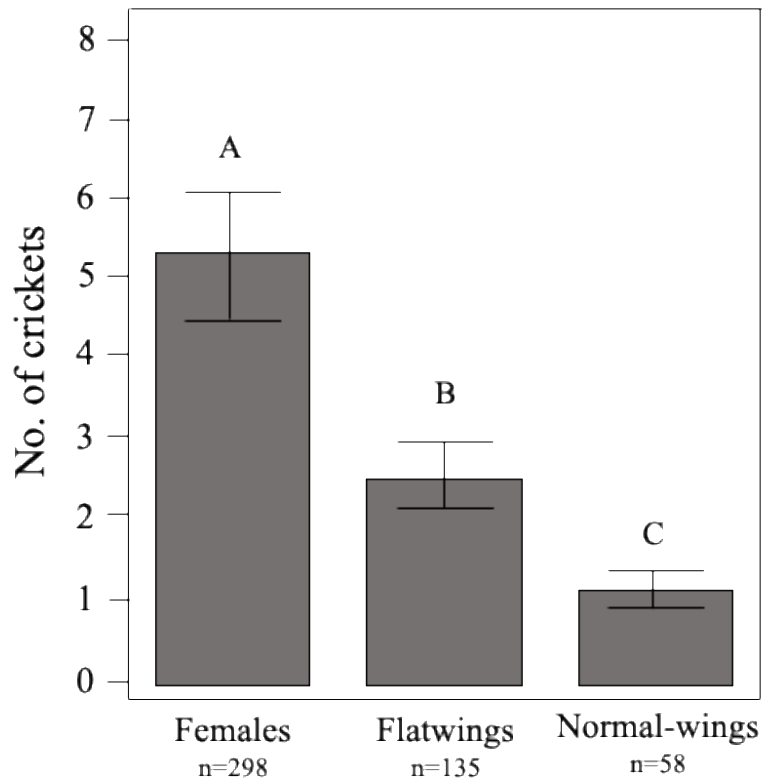
The fundamental importance of mate choice in sexual selection is well established, but the causes and consequences of variation in mating decisions are debated (Jennions and Petrie 1997; Coleman et al. 2004). Variation in female mate choice may result in variation in the form, direction, and intensity of sexual selection. In a review of 89 studies on temporally replicated estimates of selection, Siepielski et al. (2011) concluded that the sexual selection on males is stronger and more variable over time than survival selection. Such fluctuations in selection may have important evolutionary implications, such as maintaining genetic variation in sexually-selected traits (Radwan 2008; Bell 2010), or even maintaining polymorphisms in natural populations. Flexibility

in female choosiness behavior in *T. oceanicus* may be responsible for the maintenance of the normal-wing/flatwing polymorphism, but more work is needed to determine the exact mechanisms driving variation in the expression of preferences in this system.

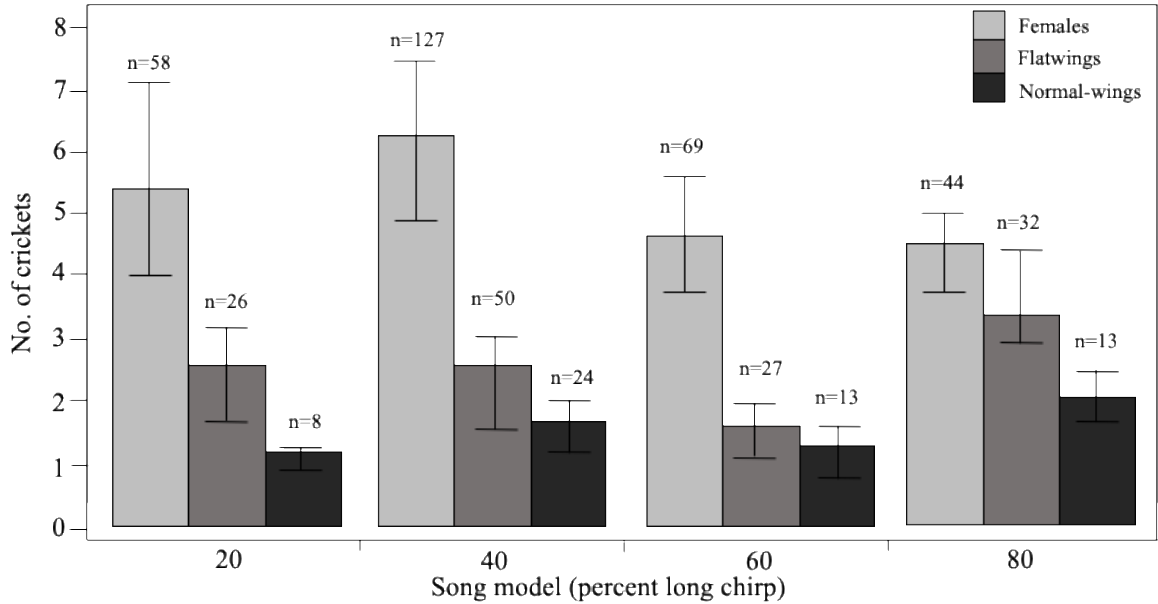
### **Acknowledgements**

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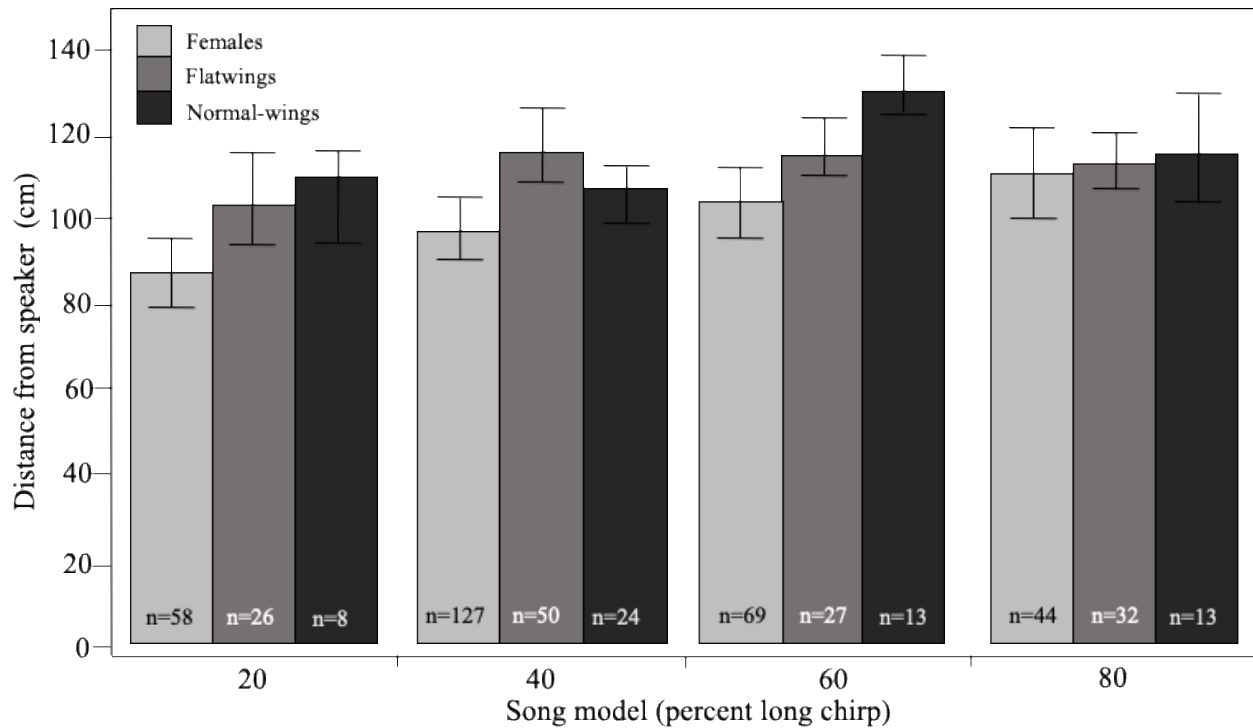
## Figures



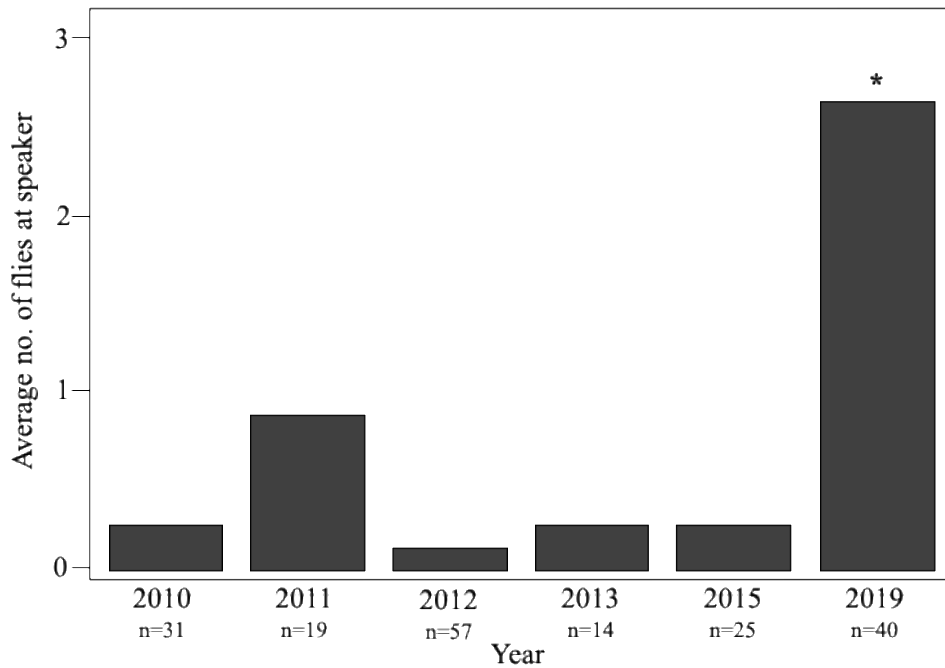
**Figure 2.1** Avg. number of crickets at the playback speaker by cricket type: female, flatwing, and normal-wing. Error bars represent  $\pm 1$  SE. We broadcast calling song models from speakers in the field. After 20-min of playback we measured the number of crickets of each type within a 2-m radius of the speaker. The different letters over the bars indicate statistically significant differences between groups. N equals the total number of crickets of each type over 11 nights sampled.



**Figure 2.2** Average number of crickets at speakers broadcasting four different calling song models. Error bars represent  $\pm 1$  SE. We broadcast calling song models varying in percent long chirp from 20%-80% from speakers in the field. After 20-min of playback we measured the number of crickets of each type within a 2-m radius of the speaker. We performed a total of 171 trials across 11 nights. N equals the total number of each cricket type at each of the four playback speakers across all nights.



**Figure 2.3** Average distance that crickets settled from a playback speaker broadcasting one of four different song models. Error bars represent  $\pm 1$  SE. We broadcast calling song models varying in percent long chirp from 20%-80% from speakers in the field. After 20-min of playback we measured the distance that each cricket settled from the speaker broadcasting the calling song model. We did not collect data on crickets that were more than 2-m from the speaker. We performed a total of 171 trials across 11 nights. N equals the total number of each type of cricket across all nights.



**Figure 2.4** Average number of flies at playback speakers per sampling event per year. We compared data collected during the summer of 2019, in which the number of flies on the playback speaker was noted after each phonotaxis trial, with the number of flies on playback speakers between the years of 2010-2013 and 2015. In 2019, *Ormia ochracea* data were collected using speakers broadcasting four song models that vary in percent long-chirp from 20%-80%. Song model did not affect the number of crickets on the playback speakers. Parasitoid fly data from previous years was collected using a single normalized song model. N equals the number of sampling events in a given year.

**Chapter 3: Interaction between opposing selective forces creates stable and unstable equilibria helping explain differential maintenance of a polymorphism across populations of the Pacific field cricket, *Teleogryllus oceanicus***

## Abstract

Not all polymorphisms are maintained in the same proportions or through the same processes across populations. Yet the processes that result in the differential maintenance of a polymorphism across populations remain largely unidentified. We examined how a novel wing polymorphism is maintained across different Hawai'ian island populations of the Pacific field cricket, *Teleogryllus oceanicus*. Here, a novel male morph, flatwing, that has lost the ability to produce conspecific calls, co-occurs with an ancestral morph, normal-wing, that has retained the ability to produce sound. Normal-wings can attract females, but may also attract an acoustically orienting parasitoid fly, *Ormia ochracea*, that can kill calling males. Flatwings evade predation from the fly, but are unable to attract females. Despite the trade-offs that each male faces, these morphs co-exist in different, relatively stable proportions across three Hawai'ian islands. Using spatially-explicit, agent-based modeling, we tested the hypothesis that an interaction between the intensity of selection against flatwings due to female choosiness and against normal-wings due to parasitoid pressure leads to differential maintenance of this wing polymorphism across Hawai'i. Stable equilibria arose from two primary conditions: (1) non-linear frequency-dependence in fitness between the morphs leading to (2) flatwing fitness exceeding normal-wing fitness as some flatwing frequencies. Variation in both female mating preferences and parasitism rates influenced relative frequencies of each wing morph at equilibrium. The non-linear frequency-dependence of male mating success necessary for equilibria to occur arises from changes in spatial dispersion of males of both types as their relative frequencies vary. The relationships that emerged between these variables shed light on how polymorphisms can be differentially



maintained across natural populations, particularly where patterns of female-male encounters lead to spatial constraints of interacting individuals.

## **Introduction**

How polymorphisms are maintained has intrigued scientists for many decades (Sandre et al. 2007; Field and Barrett 2012; Limeri and Morehouse 2014; Sowersby et al. 2015; Pintar and Resetarits 2017; Wittmann et al. 2017; Heinen-Kay et al. 2020). Often, fitness differences among individuals in different environments leads to the maintenance of alternative phenotypes as one morph is incapable of having higher fitness in all environments (Mérot et al. 2020). For example, in side-blotched lizards, *Uta stansburiana*, a polymorphism in throat color—orange, yellow, blue—with associated alternative reproductive behaviors is maintained within a single population such that each morph is capable of outcompeting only one other morph—like a game of rock-paper-scissors (Sinervo and Lively 1996). Yet, not all polymorphisms occur in the same proportions across populations. In *U. stansburiana*, the three color morphs are not found in equal proportions across their range in North America (Corl et al. 2010). In some areas, a single morph prevails, whereas all three morphs can be found in different, stable proportions across different populations (Corl et al. 2010). This is important because polymorphism loss within some populations has been associated with rapid divergence across populations, and may even aid in new species formation (West-Eberhard 1986; West-Eberhard 1989). While a lot of attention has been given to how a polymorphism is maintained within a population (e.g. Sandre et al. 2007; Field and Barrett 2012; Limeri and Morehouse 2014; Marchinko et al. 2014; Sowersby et al. 2015; Pintar and Resetarits

2017; Heinen-Kay et al. 2020; Mérot et al. 2020), less attention has been given to how polymorphisms are maintained in different proportions across different populations.

Within a population, multiple morphs are typically maintained through various mechanisms of balancing selection (Hartl and Clark 2007; Fijarczyk and Babik 2015; Gloss and Whiteman 2016). One such mechanism of balancing selection includes seasonal or temporal variation in the environment that can affect the intensity of selection on each morph (Hartl and Clark 2007). Such changes can give one morph a fitness advantage over another in the short-term that can then lead to changes in the relative proportion of each morph in the long-term (Cook and Saccheri 2013). For example, in the peppered moth, *Biston betularia*, changes in the amount of pollution throughout the Industrial Revolution led to changes in the effectiveness of the moths' crypsis and favored dark coloration who could blend with soot-covered trees while at rest (Cook and Sacheria 2013). This ability to blend with soot-covered trees allowed individuals to avoid predation (Cook and Sacheria 2013). However, as pollution decreased post-industrialization, the proportion of white to dark moths shifted in favor of a more balanced polymorphism (Cook and Sacheri 2013).

Balancing selection can also arise from a combination of opposing selective forces that each favor different phenotypes. For instance one morph can have higher survival, but weaker reproductive success, while an alternative morph can confer higher reproduction at the cost of survival (Mérot et al. 2020). Further, these types of life-history trade-offs in which fitness in one component of life-history, like survival, can trade-off with another component of life history, like reproduction, can vary spatially and temporally as well (Johnston et al. 2013; Mérot et al. 2020). If this can occur within a

population, it stands to reason that across populations, temporal and seasonal changes can result in the differential maintenance of a polymorphism.

We have an opportunity to examine how a polymorphism is maintained across populations with the recent and rapid rise of a novel wing mutation in the Pacific field cricket, *Teleogryllus oceanicus*. Male crickets signal to attract females by rubbing specialized structures on their forewings together to produce a long-range calling song that can attract females from a distance (Otte 1994). In the Hawai‘ian populations of *T. oceanicus*, the calling song also attracts an acoustically orienting parasitoid fly, *Ormia ochracea*, that use the calling song to locate hosts on which they deposit larvae (Zuk et al. 1993; Otte 1994). Larvae of *O. ochracea* burrow into the male and begin consuming his tissues (Adamo et al. 1995). Although males continue to call and remain active for several days post-infection, after about a week they die (Cade 1984; Kolluru et al. 2002). The calling song is also exploited by eavesdropping conspecific males that exhibit an alternative reproductive tactic called satellite behavior (Rowell and Cade 1993; Zuk et al. 1995). Thus, males will either signal to attract females or adopt this satellite strategy in which an individual foregoes calling, is attracted to and settles near a calling male, and attempts to intercept and mate with females who approach the calling male (Zuk et al. 1995; Shuster 2010; Taborsky and Brockmann 2010). Satellite behavior is common among chorusing animals like anurans and insects (Gerhardt and Huber 2002; Crockerbuta and Leary 2018) and has also been documented in birds (Lank et al. 2002; Küpper et al. 2016) and fishes (Tudor and Morris 2009; Neff and Svensson 2013), and can be a useful strategy for curbing the costs associated with signaling, such as higher energetic

demands (Stoddard and Salazar 2001) and the increased risks of predation and parasitism (Zuk and Kolluru 1998).

Recently, Hawai'ian populations of *T. oceanicus* have experienced major population-level shifts with the rapid rise and spread of a novel male phenotype, flatwing (Zuk et al. 2006; Tinghitella 2008). The result of a single, sex-linked mutation, flatwing males are incapable of producing conspecific calling or courtship song (Tinghitella 2008). Flatwings first appeared on Kaua'i and spread throughout the population in about 20 generations from its initial discovery (Zuk et al. 2006). The mutant flatwing males now co-exist with non-mutated, normal-wing males on three Hawai'ian islands. Although mutations in the Kaua'i and O'ahu populations share the same mode of X-linked Mendelian inheritance, they appear to be under independent genetic control (Pascoal et al. 2014). Normal-wings have retained all of the necessary wing structures for producing conspecific calling and courtship song, whereas flatwings have lost all of these specialized wing structures, and are obligately silent (Zuk et al. 2006). Where flatwings and normal-wings co-occur, both male morphs will engage in satellite behavior. Satellite behavior is employed at two levels: normal-wings who engage in a facultative satellite strategy, most likely employed to curb the costs of calling, such as the risk of parasitism; and flatwings who engage in an obligate satellite strategy as their primary means of encountering and mating with females (Tinghitella et al. 2009; Olzer and Zuk 2018). While flatwings are at a disadvantage because they cannot attract females on their own, they have been variably successful as a group. They now comprise approximately  $\geq 90\%$  of male *T. oceanicus* on Kaua'i;  $\sim 50\%$  of male *T. oceanicus* on O'ahu; and  $\sim 2\%$  of *T. oceanicus* males on the Big Island of Hawai'i (Zuk et al. 2018). Frequencies of flatwings

on Kaua‘i and the Big Island have been relatively stable, whereas their proportions at two sites on O‘ahu have been more variable (Zuk et al. 2018). We still do not know why this polymorphism is maintained in different, stable proportions across these 3 island populations. Here we use a model to ask: what might be leading to the maintenance of different proportions of flatwings across Hawai‘i and what influences its relative abundance in proportions where it fluctuates? Given the mating penalty flatwings pay relative to normal-wings, under what conditions do they persist at all?

Given the importance of female choosiness on male fitness, one possibility might have to do with differences in female mating behavior. Much empirical work about how and why flatwing has persisted has revolved around understanding the role of female choosiness on male fitness (Simmons et al. 2001; Bailey and Zuk 2008; Tinghitella and Zuk 2009). Female choosiness, which often acts counter to parasitism in its effect on male fitness, may help explain the maintenance of this wing polymorphism. In laboratory studies of *T. oceanicus*, both females from current Hawai‘ian populations with flatwings and populations without flatwings will mate with flatwings despite their lack of song (Tinghitella and Zuk 2009). However, current Hawai‘ian females from populations with the fly were more likely to mate with flatwings than were females from unparasitized populations from across the south Pacific (Tinghitella and Zuk 2009). Taken together this suggests that flexibility in female choosiness predated the flatwing mutation; however, it also suggests that there has been subsequent selection on current Hawai‘ian females to reduce their choosiness, which may have aided in the success of flatwing across the Hawai‘ian populations (Tinghitella and Zuk 2009). The relatively high (50%) rate at which Kaua‘i females showed interest in mating with flatwings compared to females

from other populations probably facilitated the spread of this wing mutation on Kaua'i (Tinghitella and Zuk 2009). However, females still rejected at least 50%, and in some cases more, of flatwings encountered, suggesting that female choosiness alone does not explain the success of flatwings.

Another possibility is that variation in the rates of parasitism and the male behavioral response of satellite behavior modulate differences in flatwing proportions across Hawai'i. Using agent-based modeling, Rotenberry et al. (2015) examined the importance of parasitism, background mortality rates, female choosiness, and population sex ratio on the propensity of normal-winged crickets to call versus act as a satellite each night. Increasing parasitism could drive the optimal male reproductive tactic from pure caller to a pure satellite strategy, although under a variety of conditions a mixed strategy prevailed even under high rates of parasitism (Rotenberry et al. 2015). This suggests that in natural populations, flatwing success is unlikely to be the sole result of parasitism on normal-wings, although it is likely to play a role. Rotenberry and Zuk (2016) examined the relative influence of various demographic, ecological, and male and female behavioral factors on reproductive success in the simulated model cricket population in more detail. They varied parasitism rates and background mortality rates; sex ratio and population density; the propensity of males to adopt a satellite strategy; and different levels of female choosiness. Here, parasitism and the number of females in the population had the largest effects on male fitness, whereas the effect of satellite behavior was the smallest overall. This suggests, again, that parasitism alone cannot explain the

maintenance of the non-calling “satellite-like” morph in natural populations. Both models used only one male morph (Rotenberry et al. 2015; Rotenberry and Zuk 2016).

Here we examine how both female choosiness and parasitism influence the relative success of flatwing. We extend the agent-based, spatially explicit model previously used to assess the effects of acoustic parasitoids on alternative mating strategies in crickets in Rotenberry et al. (2015) and Rotenberry and Zuk (2016). In our extended model we added a flatwing morph and examined its fitness (number of females mated during a individual’s lifetime) relative to that of normal-wings. In addition to varying parasitism rates, we also varied the propensity of females to mate with flatwings vs. normal-wings, based on previous work by Tinghitella and Zuk (2009) that suggests that the rate at which females mate with flatwings may have had a direct effect on the initial success of the flatwing mutation and its subsequent maintenance. In addition, we varied flatwing frequency across simulations to detect conditions under which flatwings are likely to persist despite their mating probability handicap, and to understand the proportions of flatwings in the wild.

## **Methods**

Our model is an extension of previously published models; as these are described in detail elsewhere (Rotenberry et al. 2015; Rotenberry and Zuk 2016; figure 3.1), we provide a summary of its important features. The model was created using an agent-based, spatially explicit simulation modeling environment made available through the NetLogo platform (Wilensky 1999). The spatial landscape in our extended model is comprised of a 100 x 100 grid of “patches”. Within this landscape, each agent (an

individual cricket) occupies a single patch, moves around patches, and interacts with other individuals within the model. We assign agent types— here flatwings, normal-wings, and females— and then we assign rules to each type that dictate its behavior and the outcome of interactions with other individuals based on probabilistic, not deterministic, parameters. These movements and interactions occur over 240 discrete time steps throughout a night. A step is equivalent to 3 minutes in a 12-h night. A patch then is functionally equivalent to a 1x1 meter square.

The rules that govern movement and behavior have been described in detail elsewhere (Rotenberry et al. 2015; Rotenberry and Zuk 2016), and we briefly summarize them here. At the start of each night, a male (flatwing or normal-wing) decides with some probability which mating strategy to adopt, calling or satellite. Males acting as satellites do not call and do not attract females from a distance. A satellite male is phonotactic toward calling males and will settle adjacent to a calling male that he encounters. While acting as satellites, males are not subject to parasitism. Normal-wings that call remain stationary and are detectable by females and satellites over a fixed radius of patches. As callers, normal-wings are subject to parasitism at a constant rate throughout each run. If parasitized, a normal-wing will continue following the behavioral rules assigned to him, but will die three nights later. Flatwings can either act as a “caller” or satellite; when they act as a “caller,” they are not detectable by females or satellites, and instead are stationary throughout the night. They can still be encountered by females who happen upon them while moving around the landscape. Note that despite their inability to produce an audible or detectable call (Raynor et al. 2020), flatwings will stridulate at rates similar to those of normal-wings (Olzer 2021, dissertation chapter 4).



Females are also phonotactic. A female can detect calling males if she moves within a certain radius around him and will move towards calling males with some probability of attraction. “Encounters” occur when an individual female moves into a patch that is adjacent to a patch that is occupied by a male. As an individual female moves about the landscape she can encounter satellite males and stationary flatwings. With some probability, females will mate with whomever they encounter. If a female encounters a male and decides not to mate with him, she simply continues moving throughout the space and interacting with other individuals. When not moving towards a calling male or settling adjacent to one, but females and satellite males wander randomly about the landscape.

Because of the previously observed differences in the likelihood of mating as a flatwing vs. normal-wing in empirical studies, we set mating probabilities to be unequal between the two male morphs. Flatwings always have a lower probability of mating with encountered females than normal-wings. However, the probability of mating for each morph is the same whether he is acting as a satellite or caller for the night. As a satellite, males encounter fewer females overall, which is expected to lower the overall mating success of satellite males (Rotenberry et al. 2015).

At the end of each night, the demographic data are calculated. For each individual male, his number of matings for that night are added to his number of matings from previous nights. Any normal-wings that are calling are parasitized with a certain probability, and males that were parasitized 3 nights prior “die” and are removed from the population. All crickets, regardless of sex, wing morph, or calling status, die with some probability determined by a background mortality rate. All remaining crickets (i.e.

“survivors”) age by one night and the simulation continues. To maintain constant sex ratio, morph ratio, and density throughout a run, individuals that die are replaced in kind. The simulation then repeats until the last male of the initial population dies (many replacement males have not yet lived a full life). Our metric of fitness is the average lifetime number of females mated over all males in the initial cohort for each male morph. We performed 10 runs for each combination of input variables to account for stochasticity within the model.

## **Simulations**

For the simulations, we parameterized the model using a similar range of values as in previous versions (Rotenberry et al. 2015; Rotenberry and Zuk 2016), which were based on our own investigations of Hawai‘ian *Teleogryllus oceanicus* (cited in the preceding references) and similar studies by Walker and Cade (2003) on *Gryllus texensis*, also parasitized by *O. ochracea* (Table 3.1). In this model, we held constant population density and sex ratio, nightly probability of a male following a satellite strategy, and female phonotaxis (the probability she moves towards a male she hears calling). We fixed the auditory radii (number of patches over which a female or a satellite male can detect a calling male) at 20 patches.

We manipulated 3 variables of interest: flatwing frequency, parasitism, and flatwing mating probability, (FMP), the probability that a female encountering a flatwing will mate with him. We varied flatwing frequency from 0-1 by 0.1 crossed with parasitism rates from 0-0.4 by 0.1. We implicitly varied normal-wing frequency by subtracting flatwing frequency from 1. The four values of parasitism were chosen based

on field observations of Hawaiian *T. oceanicus* (Zuk et al. 1993; Simmons and Zuk 1994; Kolluru 1999; M. Zuk, J. Rotenberry and R. Tinghitella, unpublished data). We examined the effects of flatwing frequency by percent parasitism at two levels of FMP. We choose a low value of 0.1 based on the proportion of females accepting flatwings in ancestral populations and a high value of 0.4 based on the proportion of females accepting flatwings on Kaua'i (Tinghitella and Zuk 2009). Throughout, we maintained the normal-wing mating probability at 0.5 so that flatwing mating probability was always lower.

We used locally weighted scatterplot smoothing (LOWESS; Cleveland and Devlin 1988) to empirically describe the fitness of each morph (average number of females mated in a lifetime) as a function of nightly probability of being parasitized if calling vs. flatwing frequency. LOWESS creates a nonparametric surface by computing small-scale, locally weighted regressions at points uniformly scattered over an output dataset. This allowed us to describe a fitness surface without resorting to parametric functions of the independent variables. We created surfaces with resolution of 30x30 equally scaled intervals using a tri-cubic weighting function for a locally linear estimate based on sampling a radius encompassing 10% of the total data using SigmaPlot 12.5 (Systat Software Inc 2013).

## **Results**

Our model generated 110 unique combinations of variable with 10 runs per combination. The number of females mated for flatwings was most affected by flatwing mating probability (figure 3.2). At low flatwing mating probability, 0.1, the fitness

surface for flatwings was relatively flat and hovered just under 2 females mated per individual male (figure 3.2). When flatwing mating probability increased to 0.4, a relatively high flatwing mating probability, flatwings mated with an average of 4-5 females per individual, and the fitness surface became warped and uneven (figure 3.2). This warping reflected an unexpected non-linear frequency dependence of flatwing fitness on the relative abundance of normal-wings (i.e. the inverse of flatwing frequency), and percent parasitism. As flatwing frequency increased, flatwing fitness (females mated per individual) initially increased, peaked when flatwings were about 40% of the total population of males, then declined. Percent parasitism had relatively little consistent effect on flatwing fitness (figure 3.2). Neither pattern appeared at relatively low flatwing mating probability.

The primary driver of normal-wing fitness (females mated per individual) is percent parasitism (figure 3.3). As parasitism increased, normal-wing fitness greatly suffered, such that males went from mating with an average of just under 14 females at no parasitism, to just above 4 females at 35 percent parasitism. There was an interaction between normal-wing fitness and flatwing frequency; as flatwing frequency increased (i.e. the number of normal-wings in the population declined), the average number of females mated by normal-wings increased; this increase was slightly non-linear, concave upward (figure 3.3). Unsurprisingly, flatwing mating probability had little effect on normal-wing fitness; however, there was an interaction between flatwing mating probability, and flatwing frequency such that at low rates of parasitism, as flatwing mating probability and flatwing frequency increased, normal-wing fitness decreased (figure 3.3). At a flatwing mating probability of 0.1, parasitism below 10%, and flatwing

frequency above 0.4, normal-wings achieved their highest fitness with just under 14 females mated per individual (figure 3.3). However, all else being equal, at a flatwing mating probability of 0.4, normal-wing fitness decreased to just under 10 females mated per male on average at the highest flatwing frequency (figure 3.3).

Comparing the fitness surfaces of the two morphs (figure 3.4), flatwing mating probability and parasitism rate had the greatest effect on the difference in fitness between flatwings and normal-wings. As expected, the difference in mating success between the two morphs decreases as flatwing mating probability increased and as parasitism increased (figure 3.4). While changing flatwing mating probability has little direct effect on normal-wing mating success, under conditions of high parasitism in particular, changes in flatwing mating probability affect the relative fitness of each male across a range of flatwing frequencies. These conditions of high parasitism and high flatwing mating probability allow flatwings to outcompete normal-wings (figure 3.4). At a low flatwing mating probability of 0.1, which represents a 0.4 difference in mating probability between flatwings and normal-wings, flatwings do not come close to mating with an equal or greater number of females than their normal-wing competitors, even under high rates of parasitism (figure 3.4a).

When flatwing mating probability increased to 0.4, only a 0.1 difference in mating probability between the two morphs, conditions under which flatwings and normal-wings mate with the same average number of females emerged (figure 3.4b). At a high flatwing mating probability we find conditions under which flatwings can outcompete normal-wings (figure 3.3b). Specifically, relatively high flatwing mating probability, high rates of parasitism, and across a range of flatwing frequencies, flatwings

mated with more females than normal-wings (figure 3.4b). While we expected that parasitism and flatwing mating probability would contribute to flatwing mating success, we did not expect frequency-dependence, and particularly of a non-linear type. Since this occurred even at relatively low flatwing frequencies, it suggests that the interaction between parasitism and flatwing mating probability can facilitate the establishment of flatwing from relatively few individuals in the population. Further, we found that low parasitism and high flatwing frequency resulted in the biggest differences between the fitness surfaces of flatwings and normal-wings (figure 3.4).

Most interestingly, an interaction between flatwing frequency and parasitism rate, even when flatwing mating probability was less than normal-wing mating probability, created conditions under which equilibria in the fitnesses between flatwings and normal-wings arose—particularly at higher rates of parasitism (figure 3.5). At a high flatwing mating probability (0.4), low flatwing frequency (0.2), and a high parasitism rate (0.3), an unstable equilibrium point in the fitness of flatwings and normal-wings is created. This unstable equilibrium means that changes in fitness or flatwing frequency away from the equilibrium point will cause the system to move further from equilibrium (figure 3.5a). When flatwing frequency increases to 0.6, a stable equilibrium in flatwing and normal-wing fitness is created. This stable equilibrium suggests that any changes in fitness of the two morphs or changes to flatwing frequency will push the system towards equilibrium so that flatwing frequency comes back to the equilibrium point at 0.6 (figure 3.5a). When the rate of parasitism increases to 0.4, a stable equilibrium is created at a flatwing frequency of 0.9 (figure 3.5b). The possibility of an equilibrium in fitness arose due to the

dependence of each morph's fitness on its frequency, but especially that of flatwings; the possibility of multiple equilibria arose due to the non-linearity of that dependence.

## **Discussion**

We built an agent-based model to test the hypothesis that differences in the intensity of stabilizing selection from female *T. oceanicus* and the parasitoid fly, *O. ochracea* is leading to differences in the proportion of flatwings and normal-wings across Hawai'i. Our model suggests that not only can flatwings achieve lifetimes fitness equivalent to or greater than that of normal-wings under particular conditions, the emergence of frequency dependence in mating success generates an equilibrium that allows stable persistence of the flatwing morph. Moreover, the proportion of the population consisting of flatwings at equilibrium increases as parasitism rate increases. Once beyond the lower, unstable equilibrium point, the frequency of flatwings enters a state-space that draws it towards the stable point. This scenario seems likely to have occurred on Kaua'i where there are: (1) relatively high rates of parasitism (even prior to the appearance of flatwings; e.g. Zuk et al. 1993) and (2) females mate with flatwings at a relatively high rate (i.e. flatwing mating probability is high; Tinghitella and Zuk 2009).

Although an equilibrium in flatwing frequency based on particular conditions of parasitism rate and flatwing mating probability may be stable, it is not necessarily fixed. As parasitism and mating success change, the frequency of flatwings at equilibrium changes as well. In the short term, changes in conditions are most likely to manifest as varying parasitism rates. That is, the abundance of *O. ochracea*, and the resulting rate or intensity of parasitism, can be highly variable within as between populations and seasons

(Walker and Winewriter 1991; Olzer 2021 dissertation chapter 2; M. Zuk and J. Rotenberry unpublished data). Thus, depending on local environmental conditions affecting parasitoid abundance, we may expect to see downstream effects on relative proportions of the two morphs. We suggest that this may account for the variable proportion of flatwings observed in two O‘ahu populations (Zuk et al. 2018). However, our simulation model offers no insights into how rapid changes in morph frequency might track changes in parasitism rates. Although it is certainly the case that female rates of accepting flatwings as mates may change through time, and thereby change equilibrium frequencies, we expect that this evolutionary process is likely to be slower and more unidirectional than parasitism rates drive by local environmental conditions.

Our model also offers relatively little insight as to why the flatwing mutation that appeared on the Big Island of Hawai‘i in 2010 has been slow to spread compared to other island populations (Zuk et al. 2018). Parasitoids were first detected on the Big Island in 1991. Based on dissections of individual crickets captured in the field in the early 1990s, the proportion of males parasitized on the Big Island was 15.4%, less than the 31.7% observed on Kaua‘i, but much greater than the 8% observed on O‘ahu (Zuk et al. 1993). Furthermore, female discrimination against flatwings is not significantly greater on the Big Island than on O‘ahu or Kaua‘i (Tinghitella and Zuk 2009). We suggest that perhaps flatwing frequency has not exceeded a value associated with an unstable equilibrium set by local conditions of female choosiness (i.e. probability of mating) and parasitoid abundance (i.e. parasitism rates).

It is important to note the importance of using a spatially-explicit modelling approach. The equilibria that occur depend on the non-linear frequency dependence of



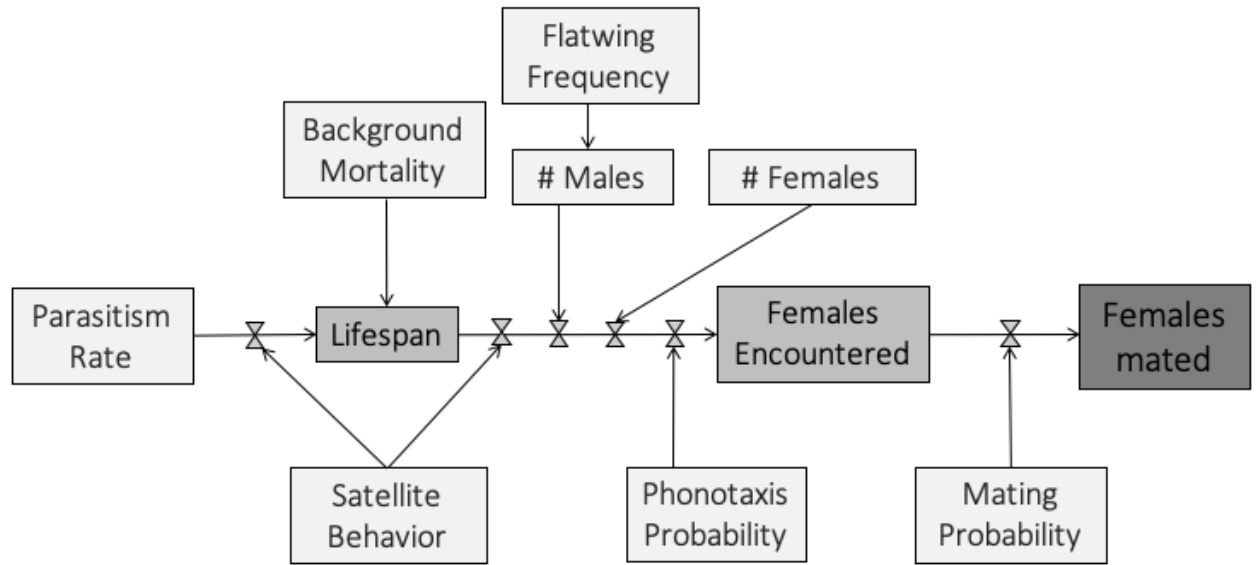
male mating success, and these dependences arise from changes in the spatial dispersion of each morph, and how females encounter them, as the frequency of each morph changes. In a previous model examining satellite behavior without flatwings, there was a slight cost to calling even in the absence of parasitoids (Rotenberry et al. 2015). This was due to an “interception effect;” callers not only attracted females, but they also attracted satellite males that may intercept and mate with females who attracted to a caller. These spatial constraints imposed by the way males and females interact can thus act to maintain polymorphisms within populations, and may be relevant to other populations or species where breeding encounters are spatially structured (e.g. chorusing anurans).

## Tables

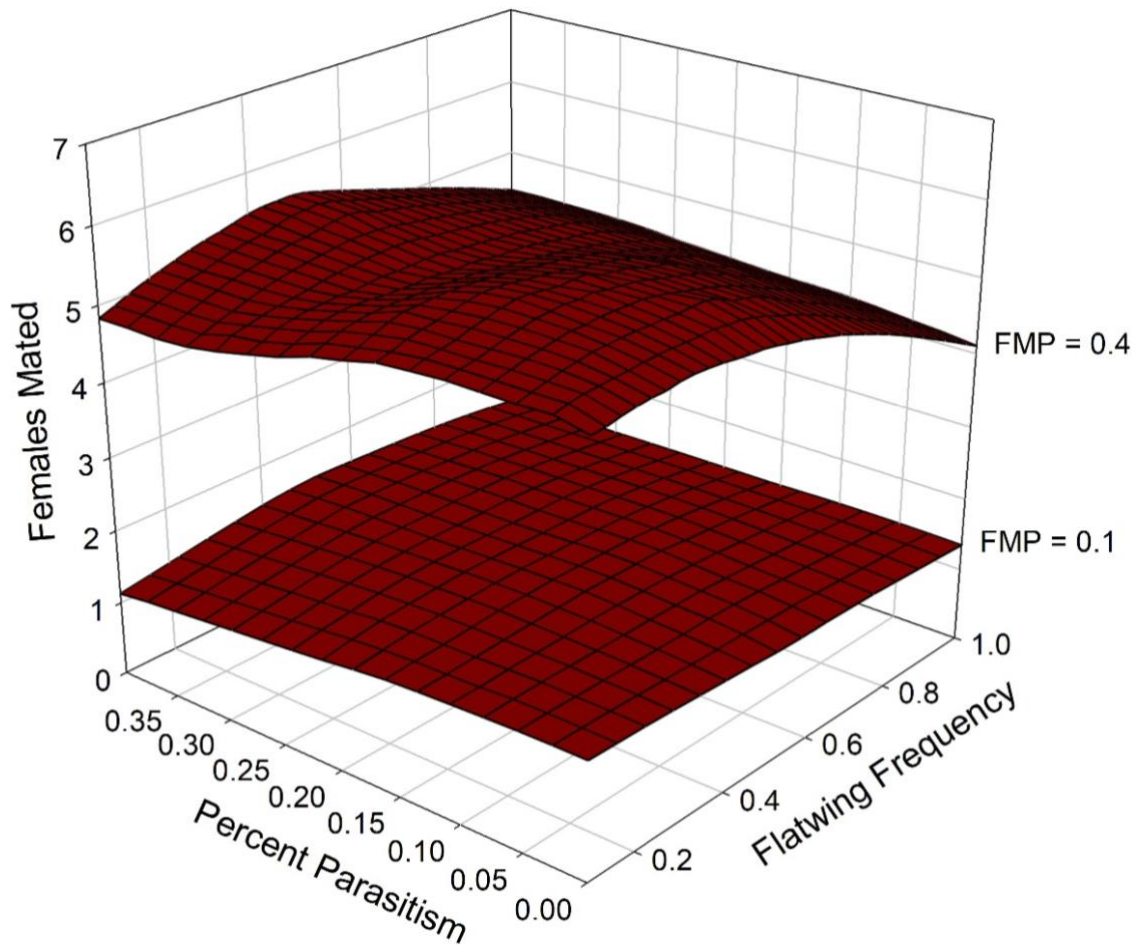
Demography			Ecology		Female choosiness			Male behavior	
Density (no. of crickets)	Sex ratio (% of females)	Flatwing frequency (% of flatwings)	Parasitism (Prob. of parasitism if NW calling)	Mortality (Prob. of nightly death)	Phonotaxis (Prob. of movement)	Mating (Prob. upon encounter)		Satellite behavior (nightly Prob.)	Auditory radius (no. of squares detectable by females)
						NW	FW		
		0							
		0.1							
		0.2							
		0.3	0						
		0.4	0.1						
100	0.5	0.5	0.2	0.5	0.5	0.5	0.1	0.5	20
		0.6	0.3				0.4		
		0.7	0.4						
		0.8							
		0.9							
		1							

**Table 3.1:** Summary of initial input values for model simulations. We varied flatwing frequency (% of males that are flatwings), parasitism rate, and flatwing mating probability, while maintaining all other model variables. FW= flatwing; NW= normal-wing. This model generated 110 combinations of variable with 10 runs per combination.

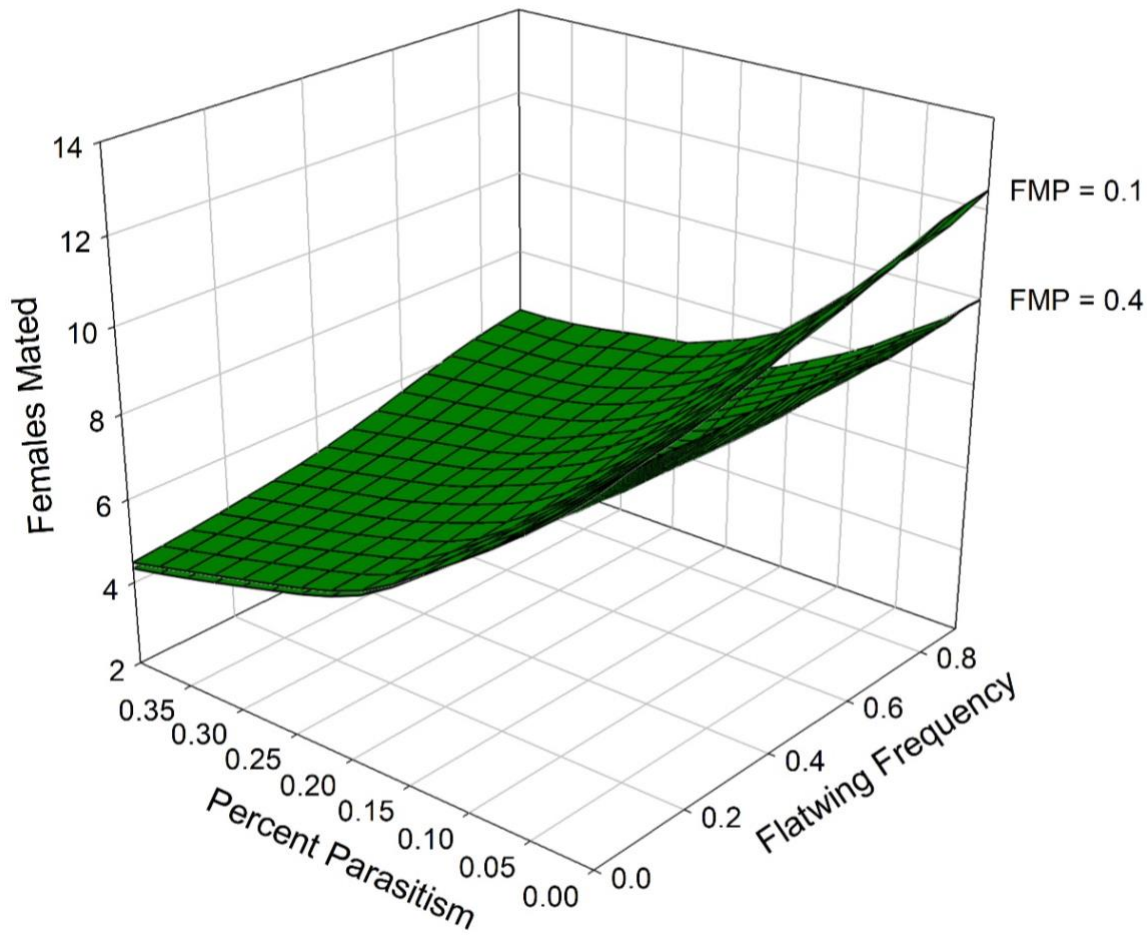
## Figures



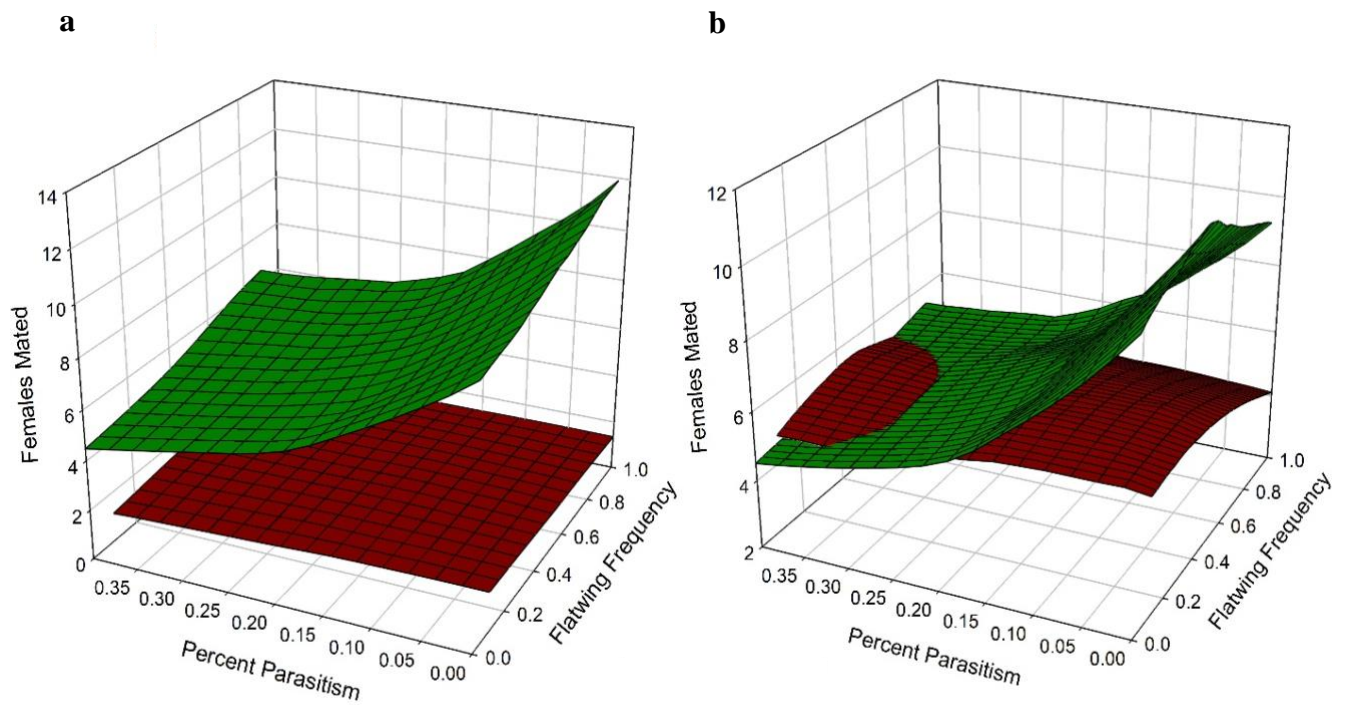
**Figure 3.1** Relationships among model variables. Light boxes are input variables, gray boxes are latent variables, and the dark box is the output variable. We varied parasitism rate, mating probability and flatwing frequency; other variables remained constant across all runs. The variable of interest is the number of females mated, a proxy for fitness in our model.



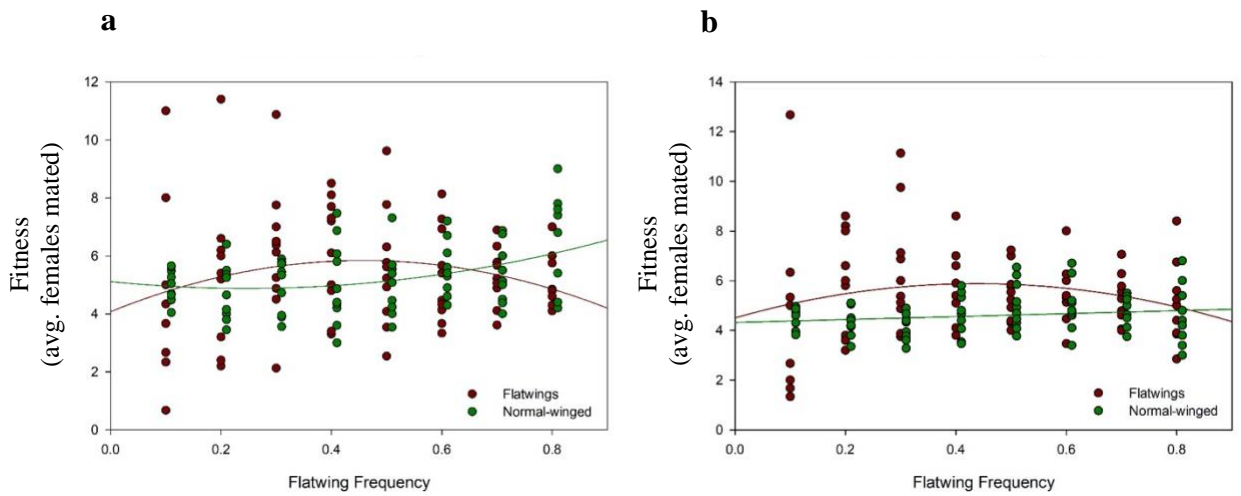
**Figure 3.2** Flatwing fitness (average number of females mated over a lifetime) as a function of parasitism rate and flatwing frequency across 2 levels of flatwing mating probability (FMP). Surfaces were fitted using LOWESS. Flatwing mating success was greatly affected by the probability of mating with females they encountered. FMP interacts with flatwing frequency and parasitism, most notably at a high level of FMP. Curvature of the upper surface illustrates the non-linear frequency dependence of flatwing fitness.



**Figure 3.3** Normal-wing fitness (average number of females mated across a lifetime) as a function of parasitism rate and flatwing frequency across 2 levels of flatwing mating probability (FMP). Surfaces were fitted using LOWESS. Flatwing mating probability had little effect on normal-wing fitness. Normal-wing fitness was most affected by parasitism rates. However, there is an interaction between parasitism and FMP, such that normal-wing fitness is highest when FMP and parasitism are relatively low. Slight non-linear flatwing frequency dependence of fitness can be seen at high percent parasitism. Note that females mated axis scale differs between fig. 3.3 and figure 3.2.



**Figure 3.4** Fitness surfaces for flatwings and normal-wings at (a) low FMP and (b) high FMP. These are the same surfaces as in figure 2 and 3. Flatwing fitness represented in red, while normal-wing fitness is represented in green. At low FMP, normal-wings are better competitors, with higher average fitness overall. At high FMP, normal-wings still do better most of the time; however, at high rates of parasitism the 2 fitness surfaces intersect, showing that there are conditions under which flatwings have higher fitness than normal-wings. Note that females mated axis scale differs between the two panels.



**Figure 3.5** Cross-section of the fitness surface in figure 3b at 0.35 and 0.4 parasitism. Cross-sections revealed stable and unstable equilibria in male fitness at relatively high parasitism rates. (a) When FMP was high, and the probability of parasitism was 0.3, an unstable equilibrium was created at low flatwing frequencies. A stable equilibrium was created at higher flatwing frequencies. (b) When we increased the probability of parasitism to 0.4, a stable equilibrium was created at much higher flatwing frequencies. Both of these graphs suggest that the 2 morphs can coexist in stable equilibria when certain conditions are present. Lines in each panel are fitted quadratic curves.

**Chapter 4: Differences in diel distribution of calling between two morphs of the Pacific field cricket, *Teleogryllus oceanicus*, suggests relaxed selection on vestigial behavior**



## Abstract

Studying behavioral vestigiality can help shed light on a longstanding question in evolutionary biology about the role of behavior in evolution and whether behavior impedes or promotes species evolution. We studied behavioral vestigiality in the Pacific field cricket, *Teleogryllus oceanicus*. In Hawai‘i there are two male morphs: normal-wings who have retained the ability to produce conspecific calling songs for attracting females; and mutated flatwings who have lost the morphological wing structures for producing sound, but continue to express the stereotyped wing movements that traditionally lead to song production in other crickets, including their non-mutated counterparts, normal-wings. We tested the hypothesis that flatwings are experiencing relaxed selection from acoustically orienting predators and female crickets-- leading to differences in singing behavior between the two morphs. Crickets will typically switch between periods of calling and rest throughout the night. We compared how the two male morphs entrain their calling effort across a 24-hour period. Normal-wings confine their calling behavior to the dark part of the diel period, whereas flatwings move their wings during the light part of the diel period more than normal-wings do. This suggests that flatwings may be experiencing relaxed selection, while normal-wings experience ongoing balancing selection, leading to differences between the morphs in calling effort across a 24-hour period.

## Introduction

A central prediction of evolutionary theory is that sometimes complex traits will become reduced or disappear completely (Porter and Crandall 2003). A major consequence of trait loss is the retention of structures or attributes that have lost their original function (Müller 2002). These traits, known as vestigial traits, are common in a variety of taxa and include: the loss of functional wings in island dwelling birds (Slikas et al. 2002; Kirchman 2009; Wright et al. 2016); the coccyx bone in humans (Tubbs et al. 2016); and hindlimbs in whales (Bejder and Hall 2002; McGowen et al. 2014) and snakes (Palci et al. 2020).

While examples of morphological vestigiality are relatively common, examples of vestigial behaviors are rare, though some exist. For example, the Palmar Grasp reflex in which human infants exercise unusual grip strength in response to mechanical stimuli has long been considered a primitive and largely unnecessary behavior for modern humans (Allen and Capute 1986), but evolved from ancestral primates whose offspring needed a firm grasp of their mothers' fur while being carried (Dewey 1935). Domesticated dogs will engage in vestigial behaviors like turning in a circle before lying down, presumably to trample down a bed of grass consistent with their ancestral environment (Irvine 2004). Many species of whiptail lizards reproduce asexually, yet they will often engage in courtship and mating behavior collectively referred to as pseudocopulation that resembles the behaviors of closely related, sexually-reproducing species. For example, in the whiptail lizard, *Aspidoscelis uniparens*, behaviors associated with pseudocopulation appear to be an ancestral trait inherited from the sexually-reproducing species, *Cnemidophorus inoratus* (Woolley et al. 2004). This suggests that pseudocopulation may

be an example of behavioral vestigiality. In both these examples of behavioral vestigiality, morphological trait loss was not a part of the evolutionary process. Sometimes vestigial structures and vestigial behaviors can coexist. For example, cormorant birds engage in a basking behavior in which, because their wings lack waterproof plumage, when their wings become wet they will spread and dry them in the sunlight (Sellers 1995). This basking behavior is not performed for thermoregulation nor is it for digestion (Sellers 1995). Instead, this behavior appears to be solely for drying, and thus lightening the weight of the wings in preparation for flight (Sellers 1995). However, the flightless cormorant, *Phalacrocorax harrisi*, need not dry their wings in preparation for flight, but do so anyway (Sellers 1995). Thus, *P. harrisi* demonstrate not only conspicuous vestigial structures (i.e. their wings), but also a vestigial behavior (i.e. basking behavior), neither of which appear to be necessary for their survival. What happens when morphological trait loss occurs alongside the persistence of behaviors related to the functional loss of a trait? Since contemporary examples of morphological trait loss are rare, it can be difficult to study the evolutionary consequences of trait loss and behavioral vestigiality. Yet, studying behavioral vestigiality can help shed light on a longstanding question in evolutionary biology about what the role of behavior is in evolution and whether behavior impedes or promotes species evolution. Furthermore, studying the causes and consequences of behavioral vestigiality may help shed light on why these traits are so rare in nature.

We have an unique opportunity to study behavioral vestigiality with the loss of a sexual signal in the Pacific field cricket, *Teleogryllus oceanicus*. Native to Australia and found on islands throughout the south Pacific, including the Hawai'ian Islands, male *T.*

*oceanicus* signal to attract females by stridulating--rubbing specialized structures on their forewings together. While stridulating, males typically produce a long-range calling song that not only attracts conspecific females for mating, but on the islands of Hawai'i, this song also attract an acoustically orienting parasitoid fly, *Ormia ochracea* (Otte 1994; Zuk et al. 1993). Gravid *O. ochracea* females locate calling males, deposit free-moving larvae around them that then burrow inside, develop within, and eventually emerge from the male, killing him in the process. In 2003, a novel male morph, flatwing, was discovered in Hawai'i (Tinghitella 2008; Zuk et al. 2006). Flatwings carry a genetic mutation that greatly reduces the wing structures necessary for producing conspecific song and renders them obligately silent (Tinghitella 2009; Pascoal et al. 2014). Flatwings are protected from the fly, but cannot attract females. Nonetheless, flatwings have been quite successful and can be found in different proportions across three of the Hawai'ian Islands (Zuk et al. 2018).

Although flatwings are incapable of producing sound, it is not for lack of trying. Flatwings continue to express the stereotyped wing movements that traditionally lead to song production in other crickets, including normal-wings. The wing movements of flatwings are identical to those of normal-wings (Schneider et al. 2018), yet the greatly reduced sound-producing wing features of flatwings resonate at highly variable acoustic frequencies that fall well outside the normal range for *T. oceanicus* (Bailey et al. 2019). Thus, flatwing stridulation does not appear to serve the ancestral purpose of attracting females, and is indeed a vestigial behavior, as suggested by Schneider et al. (2018). Furthermore, the resonant frequencies of flatwings are much higher than the optimal frequency at which *O. ochracea* detect cricket calling (Robert et al. 1996; Bailey et al.

2019). This suggests that flatwings are also not subject to selection from the parasitoid fly.

When there is strong selection on a trait from a known source, like a predator or parasite, or strong female choosiness, a prediction for trait evolution often follows directly from this (Endler 1986). However, when such a strong source of selection is removed, often no clear predictions emerge about the exact ways that evolution will proceed (Lahti et al. 2009). Because flatwing stridulation does not attract females or natural enemies, we expect selection on patterns of that stridulation to be relaxed. Here, we explore the possible consequences of such relaxed selection.

Given the energetic costs of signaling in many animals (Gillooly and Ophir 2010; Stoddard and Salazar 2011) including crickets (Prestwich 1994; Hoback and Wagner Jr. 1997; Erregger et al. 2017), we might expect to see relaxed selection manifested in calling effort. In field crickets, calling effort can be measured as time spent stridulating, which appears to be a particularly evolvable trait. Females strongly prefer males that sing more (Cade and Cade 1992; Crnokrak and Roff 1998; Holzer et al. 2003). Yet, calling is condition-dependent (Judge et al. 2008) and may trade-off with other life-history components (Hunt et al. 2004; Drayton et al. 2010; Houslay et al. 2017), like lifespan (Verburgt et al. 2011) and immunity (Kerr et al. 2010; Simmons et al. 2010). Thus, crickets will moderate their calling effort in response to various selective pressures. For example, in *Gryllus integer*, a cricket that co-occurs with *O. ochracea*, individuals exhibit an overall reduction in time spent calling, compared with other field crickets that do not experience selection from the parasitoid fly (Cade and Wyatt 1983). Similarly, in *Gryllus ovisopis*, another cricket that co-occurs with *O. ochracea*, males possess the

morphological structures for producing sound, but are behaviorally silent—similar to flatwing *T. oceanicus* (Gray et al. 2018). While flatwings do not pay a penalty from the parasitoid fly, they could still pay an energetic cost to moving their wings. Thus, a behavioral strategy in which they move their wings less or not at all may be beneficial for flatwings, as they appear to gain no fitness benefit from stridulating (Bailey et al. 2019). However, flatwings and normal-wings from the same population spend the same amount of time stridulating and in populations with flatwings, males spend the same amount of time moving their wings as populations of crickets without flatwings (Rayner et al. 2020).

While total time spent stridulating is important, it is not the only relevant measure of calling effort. Most cricket species call at night, but due to the energetic demands (Prestwich 1994; Hoback and Wagner Jr. 1997; Reinhold 1999), and other previously mentioned life-history trade-offs (Houslay et al. 2017; Drayton et al. 2010; Hunt et al. 2004; Verburgt et al. 2011; Simmons et al. 2010), continuous calling is not a feasible strategy for attracting mates. Instead, crickets will typically switch between periods of calling and rest throughout the night (Kolluru 1999). Given this, it is of interest how insects entrain their calling effort across a 24-hour period. Like other organisms, crickets possess an endogenous circadian clock (Loher 1972; Abe et al. 1997). The endogenous clock underlying circadian oscillation is maintained by an approximate 24-hour cycle of various physiological process and is an adaptation that enables organisms to respond to their external environment (Yerushalmi and Green 2009). For example, in response to changes in predator and parasite abundance in the environment, animals can respond by shifting the timing of signaling to periods when predators and parasites are less active

(Bertram et al. 2004; Vélez and Brockmann 2006). In *T. oceanicus* populations that co-occur with *O. ochracea*, males confine their calling activity to full darkness, when the fly is less active, compared to males from populations without the parasitoid (Zuk et al. 1993). Specifically, males exhibit more abrupt onset and cessation of calling at dusk and dawn when parasitoids are most active (Zuk et al. 1993). Since flatwings are not subject to such selection from parasitoids, they may not be exhibiting such shifts in the diel distribution of their calling effort.

If the two male morphs experience differences in the intensity of selection on calling behavior, than we should expect there to be measurable differences in certain attributes of calling behavior. Here we examined how *T. oceanicus* portion their calling across a 24-hour period. We tested the hypothesis that there is relaxed selection on flatwings, resulting in measurable differences in stridulatory behavior between the morphs. To test this hypothesis, we observed individual stridulatory effort across a 24-hour period. We recorded individual males for 24-hour periods, with a 12:12 photo-reversed light-dark cycle to simulate natural light cycles in the wild. We compared stridulatory behavior between flatwings and normal-wings by analyzing: (1) total time spent stridulating; (2) time spent stridulating in the light vs. dark hours; (3) the distribution of stridulating across 24-hours; and (4) among-individual variation in total proportion of stridulating. We predicted that if flatwings do not pay a survival cost imposed by the parasitoid fly, than they would be more likely to move their wings throughout the 24-hour period, and during both the light and dark hours. We predicted that normal-wings would confine their wing movements to the dark hours, as they are more likely to suffer detection, and subsequently parasitization, if they call outside of that

range. We also predicted that if there is indeed relaxed selection on flatwings, then there would be differences in the amount of among-individual variation between the two morphs.

It is not just how calling is distributed throughout the night, but how that distribution might change over an individual's lifespan. Age affects calling effort in several cricket species (Jacot et al. 2007; Judge et al. 2008; Fitzsimmons and Bertram 2011; Verbugt et al. 2011). In some crickets, there is an increase in calling effort from young to middle age (Kuriwada and Kasuya 2011). In others, calling effort is positively correlated with longevity such that males that invested more in calling effort over their lifetime, also lived longer (Fitzsimmons and Bertram 2011). In *T. oceanicus*, the fine-scale properties of the calling song do not vary with age (Walker and Cade 2003). However, we might expect an interaction between age and wing morph. Since normal-wings are subjected to parasitism, we expect that they have a decreased lifespan compared with flatwings (Zuk et al. 2006). A decreased lifespan means that normal-wings may encounter fewer females throughout their life. Normal-wings may invest more in calling effort earlier in life to compensate for the potential decrease in female encounter rates. If flatwing stridulation does not affect reproductive fitness and they are experiencing relaxed selection than we should expect more variable calling effort throughout their lives. We examined the relationship between age, morph, and calling effort. If normal-wings are compensating for a decrease in lifespan by increasing calling effort early in life, that could help explain how normal-wings balance the trade-off between parasitism risk and mate attraction.



## Methods

### (a) Study Organism

We used laboratory-reared and maintained colonies of *T. oceanicus*. We constructed lines that consisted of males descended from the Kaua‘i population. Since flatwing is the result of a single sex-linked gene (Tinghitella 2008), we used colonies of crickets that breed true for either the flatwing or normal-wing allele (Heinen-Kay et al. 2019). The Kaua‘i laboratory colony from which these lines were constructed from was established in 2003 after the discovery of flatwing. Since their creation, the normal-wing and flatwings colonies have been maintained in the same way as the general laboratory colonies, except that adult males from the general Kaua‘i laboratory colony were added to the appropriate wing morph colony every few months to maintain genetic variation and ensure that the normal-wing and flatwing colonies reflect the wild Kaua‘i populations. Laboratory colonies consist of approximately 100 adults at all times. The flatwing and normal-wing colonies have bred true for their respective wing morph since 2016. Because it is unlikely that linkage disequilibrium could have built up in the short amount of time between when the colonies were established in 2016 and when the data were collected in 2019, we assume that any observed differences in male behavior between the colonies is the result of possessing the flatwing or normal-wing allele (Heinen-Kay et al. 2019). For a full description of how flatwing and normal-wing colonies were created see Heinen-Kay et al. 2019.

Crickets in our study were reared in Caron Insect Growth Chambers (model 6025) that maintain 26°C, 75% humidity and are maintained on a 12:12 photo-reversed light-dark cycle. During early rearing, crickets are housed within 15-L plastic containers with

ad-lib access to food, water, and shelter. We checked these containers for newly emerged adult crickets twice daily. After eclosion, males were placed into individual 118-mL plastic cups with food, water, and shelter, to keep track of adult age and ensure that males remained unmated throughout the study.

In the field individuals can live at least 17 days post-eclosion (Simmons and Zuk 1994); therefore, we tested individuals that were between the ages of 6 to 16 days post-eclosion. We tested 27 flatwings and 41 normal-wings. Laboratory colonies consist of approximately 100 adults at all times.

#### **(b) Collection of sound files**

We used substrate recording to measure stridulation rates in flatwings and normal-wings. Sound is produced when an object vibrates, creating a pressure wave. This pressure wave causes particles in the surrounding medium (air, water, or substrate) to vibrate. The vibrating particles ripple outwards, vibrating nearby particles, transmitting the sound further through the medium. In acoustically communicating animals, vibrations are typically carried through the air which allows us to record sound using a traditional condenser microphone.

In our system, flatwings stridulate at such a high frequency (Bailey et al. 2019) that they cannot effectively communicate via airborne acoustic signals. Even though flatwings cannot produce an airborne acoustic signal, they still produce vibrations from the movement of their wings. At higher resonance frequencies sound degrades through the air at a much higher rate than when transmitted through a substrate. Vibrations can be detected either from appendages transmitted through a substrate or via acoustical waves

coupled with a substrate. We used contact microphones embedded in the base of a plastic recording chamber to collect sound files from both flatwings and normal-wings. For flatwings the vibrations from their wings were transmitted through the substrate and for normal-wings the acoustic signal coupled with the substrate. The testing chamber measured 8 cm by 2.5 cm by 5 cm and was connected to a Roland R-26 portable recorder for collecting waveform files.

The testing chamber and recorder were placed in a larger 52-L plastic container measuring 58 cm by 41 cm by 31 cm. We affixed 4 lights to the container that were programmed on a 12:12 photo-reversed light-dark cycle to simulate natural light cycles. We then recorded individual virgin males for 24-hours.

Between trials, the testing chamber was wiped clean with isopropyl alcohol to eliminate any scent from previously tested males. While in the chamber, males were given access to food, water, and shelter ad-libitum. Sound files were collected from January to May 2019.

### **(c) Detecting signal from song files**

Audio recordings were processed with a detection algorithm to automatically identify periods of calling and silence. All processing was performed in Python and code is available for download. [<https://github.umn.edu/hbraun/thicc-detector>]. Audio was first preprocessed to whiten the data and reduce noise. An autoregressive (AR) model was estimated from a short negative (no chirp) audio example and the inverse of this filter was used to whiten the input data. An AR model of order 1280 was used; this large filter order was necessary to accurately model a low-frequency hum caused by the audio

recorder's DC power supply. All template generation and detection was performed with this whitened audio.

We generated two spectrogram-based detection templates from positive (chirp present) examples of normal-wing and flatwing audio, respectively. Training examples of individual chirps were manually cropped from a short audio sample and averaged to generate a composite chirp signal. Each new training example was synchronized with previous ones by maximizing its cross-correlation with the running average of previously considered examples. We then generated a spectrogram template from this composite signal. Detection was performed by cross-correlation of the recording's spectrogram with the template. The resulting signal was thresholded and binary opening and closing operations were applied to convert individual chirp detections to continuous regions of calling and silence. Lastly, we generated a summary statistic of percentage of time spent calling for each minute of audio and this was then used for further analyses.

#### **(d) Analysis**

We performed a Generalized Linear Mixed Model (GLMM) with the proportion of stridulating as the response variable; morph (normal-wing vs. flatwing), photoperiod (dark vs. light), and age as fixed effects and a random effect of individual cricket. We also included interaction terms of morph by photoperiod and morph by age as fixed effects.

To examine whether the variance in proportion of time spent stridulating among individuals of each wing morph differed, we also conducted an Analysis of Means

(ANOM) Variances–Levene’s test. We used the proportion of calling as the response variable and morph as the fixed effect.

We used JMP Pro v. 14 for all data analyses.

## Results

Males stridulate significantly more in the dark than in the light (figure 4.1; GLMM, photoperiod:  $F_{1,67}=102.24$ ;  $p<0.0001^*$ ). Occasionally, males move their wings during the light hours, but flatwings move their wings during the light hours significantly more than normal-wings (figure 4.1 and 4.2; GLMM, morph\*photoperiod:  $F_{1,67}=10.03$ ;  $p=0.0023^*$ ). Flatwings stridulate during the dark 27.7% of the time and 6.78% of the time in the dark. Whereas normal-wings stridulate 43.2% of the time in the dark and 0.5% of the time in the light.

Normal-wings stridulate slightly more than flatwings (23.6% of the time vs. 18.1%) but, this difference was not significant (figure 4.1; GLMM, morph:  $F_{1,65}=2.14$ ;  $p=0.15$ ).

The variances in the proportion of stridulating between flatwings and normal-wings did not differ (figure 4.3; ANOM–Levene’s test:  $F_{1,67}=0.09$ ;  $p=0.77$ ).

Age did not have a significant effect on stridulation effort (GLMM, age:  $F_{1,65}=1.28$ ;  $p=0.26$ ), and there was no interaction between age and morph (GLMM, age\*morph:  $F_{1,65}=0.29$ ;  $p=0.59$ ).

## Discussion

Consistent with the idea that flatwings experience relaxed selection on their stridulation behavior, flatwing crickets stridulate more than normal-wings during the light period. This finding is in line with previous observations that *T. oceanicus* shift their calling effort such that they have a more abrupt onset and cessation of calling during times when the parasitoid fly is most active, primarily dusk and dawn (Zuk et al. 1993).

Given the metabolic demands associated with calling (Erregger 2017; Hoback and Wagner 1997; Prestwich 1994), another place we might expect to see relaxed selection manifest is through calling effort measured as total time spent moving the wings. However, there were no differences in total time spent stridulating between the morphs, consistent with Rayner et al. (2020). While flatwings experience relaxed selection from the parasitoid, they may still pay a penalty to moving their wings in the form of an energetic cost. Even though flatwings are moving their wings, the costs may not be equal to normal-wings. One possibility is that flatwings expend less energy when stridulating. This could be due to differences in the inertia associated with opening and closing the forewings during stridulation (Bennet-Clark 1989). Crickets produce sound as their forewings close and specialized structures like file and scraper engage with each other (Bennet-Clark 1989). During stridulation the scraper, a sclerotized region on the forewing, is gathered and released by the successive teeth on the file (Bennet-Clark 1989). The rate of this catch-and-release equals the frequency of the sound produced by the cricket, known as the carrier frequency,  $f_c$ . The friction and power of these wing movements is directly related to the rate of metabolism. The file and scraper are greatly reduced and nearly absent for flatwings (Pascoal et al. 2014; Zuk et al. 2006). As such,

we might expect that flatwings generate less friction, and thus less energetic output, when they stridulate. Prestwich and O'Sullivan (2005) developed a technique in which they related the minor morphological differences of the stridulatory structures between two sister species of mole crickets to acoustic power and metabolic output (Prestwich and O'Sullivan 2005). A similar technique could be applied to *T. oceanicus* to examine whether there are metabolic differences between the morphs.

Surprisingly, age did not affect stridulatory behavior in *T. oceanicus*. This is unexpected because age differences in calling effort have been reported in a number of cricket systems. For example, in spring field crickets, *Gryllus veletis*, male calling changed with age such that as males got older they decreased their calling effort (Fitzsimmons and Bertram 2011). In a longitudinal study of male *Gryllus pennsylvanicus*, acoustic parameters changed with age such that older males produced calls with more pulses per chirp and shorter pulse durations (Judge 2011). Similarly, in *Gryllus bimaculatus* nearly all song traits changed as male aged, indicating a general slowing down of wing movements in older males (Verburgt et al. 2011). Lastly, in *Gryllus campestris*, pulses per chirp increased and carrier frequency decreased as males aged (Jacot et al. 2007). These studies reveal that the fine-scale structure of male calls may reliably change as males age. This is important because sexually-selected traits are often thought to convey information about male quality and condition (Zahavi 1977; Hamilton and Zuk 1982), both of which can change with age (Rezaei et al. 2015; Houslay et al. 2017). Indeed, changes in female preferences based on age have been documented in a number of taxa (Kokko and Lindstrom 1996; Beck and Promislow 2007; Rezaei et al.

2015; Rodríguez-Munoz et al. 2019). Yet, evidence that males directly signal their age can be inconclusive (see Brooks and Kemp 2001 for a review).

In many of the above examples we see that the fine-scale properties of cricket song are subject to selection. Many of the fine-scale properties of signals are used by females for species recognition (Garcia et al. 2020) and to assess male quality (Fitzsimmons and Bertram 2011; Zhemchuzhnikov et al. 2017; Garcia et al. 2020). In *T. oceanicus*, male calling song is comprised of a series of trills, called the long-chirp, followed by a series of staccato short-chirps. The proportion of long- to short-chirps in the calling song is particularly important in female mate choice (Simmons 2004; Simmons et al. 2001). Females respond fastest to calling song models with 60% long chirp (Bailey and Zuk 2008). This means that these fine-scale properties of calling song are under selection from females. Further, flatwings who engage in a satellite strategy in which they associate with calling males and attempt to intercept females, also exhibit a preference for song models containing 60% long chirp (Olzer and Zuk 2018). Thus, calling song may be under stabilizing selection from eavesdropping males (Olzer and Zuk 2018) and from females. Oceanic island crickets have longer long chirp elements and shorter short chirps compared with ancestral, mainland Australian crickets (Zuk et al. 2001). Among the oceanic islands, Hawai'ian crickets have shorter songs overall and shorter intervals within songs, compared with other island populations (Zuk et al. 2001). This is important because it demonstrates that the fine-scale properties of the calling song are capable of changing in response to selection, as the Hawai'ian populations experience strong opposing selection, compared with the other south Pacific populations (Zuk et al. 2001). Given that, it stands to reason that a lack of selection from the parasitoid on



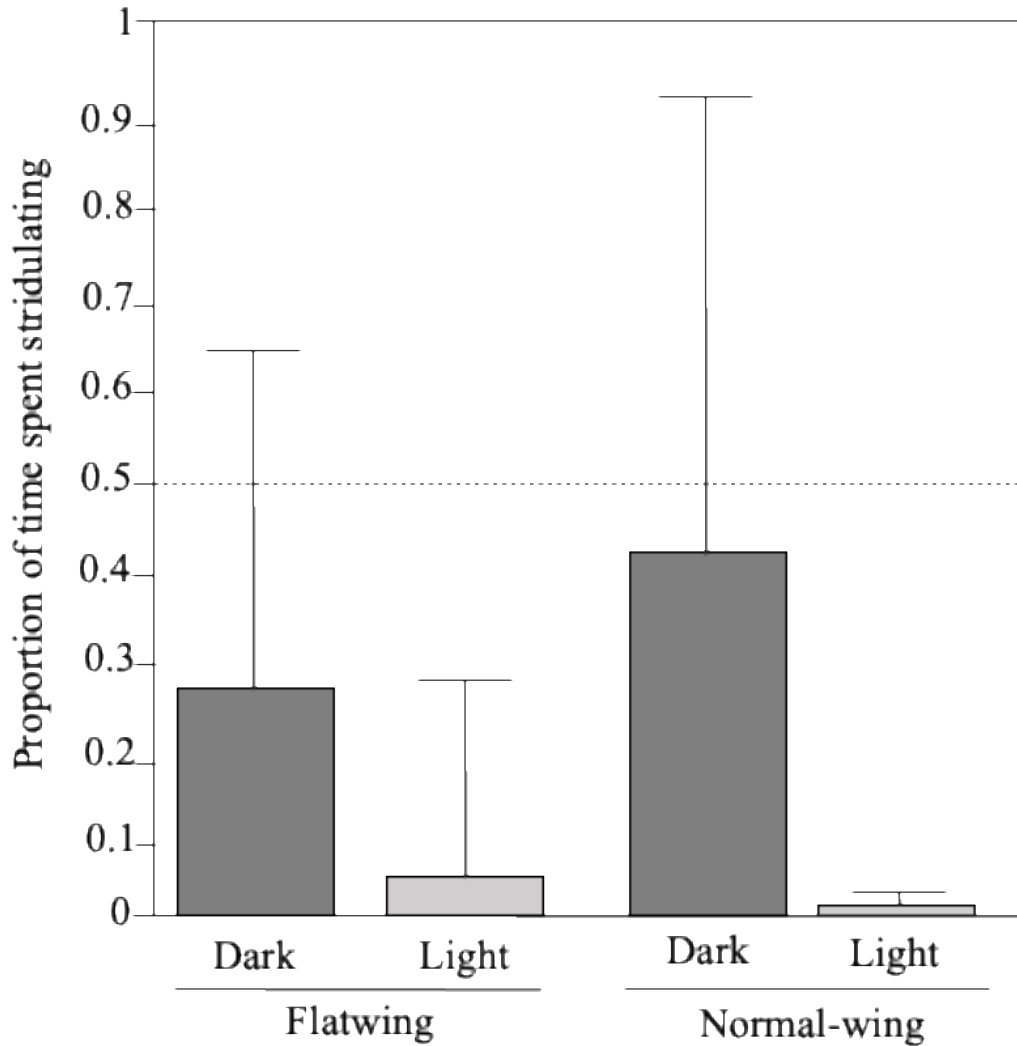
flatwings, could mirror the results of Zuk et al. (2001), in which populations without the parasitoid differed in these fine-scale properties of calling song compared with the Hawai‘ian populations where the threat of parasitism looms large. It is possible that the vestigial wing structures of *T. oceanicus* could be co-opted and elaborated via sexual selection. Recent work revealed that female *T. oceanicus* from a population of Hawai‘ian crickets preferentially associated with attenuated acoustic stimuli produced by some flatwing males, compared with silence (Tinghitella et al. 2018). Further work could examine the fine-scale properties of *T. oceanicus* calling song to examine whether relaxed selection is manifesting as differences in these properties between flatwings and normal-wings.

## Tables

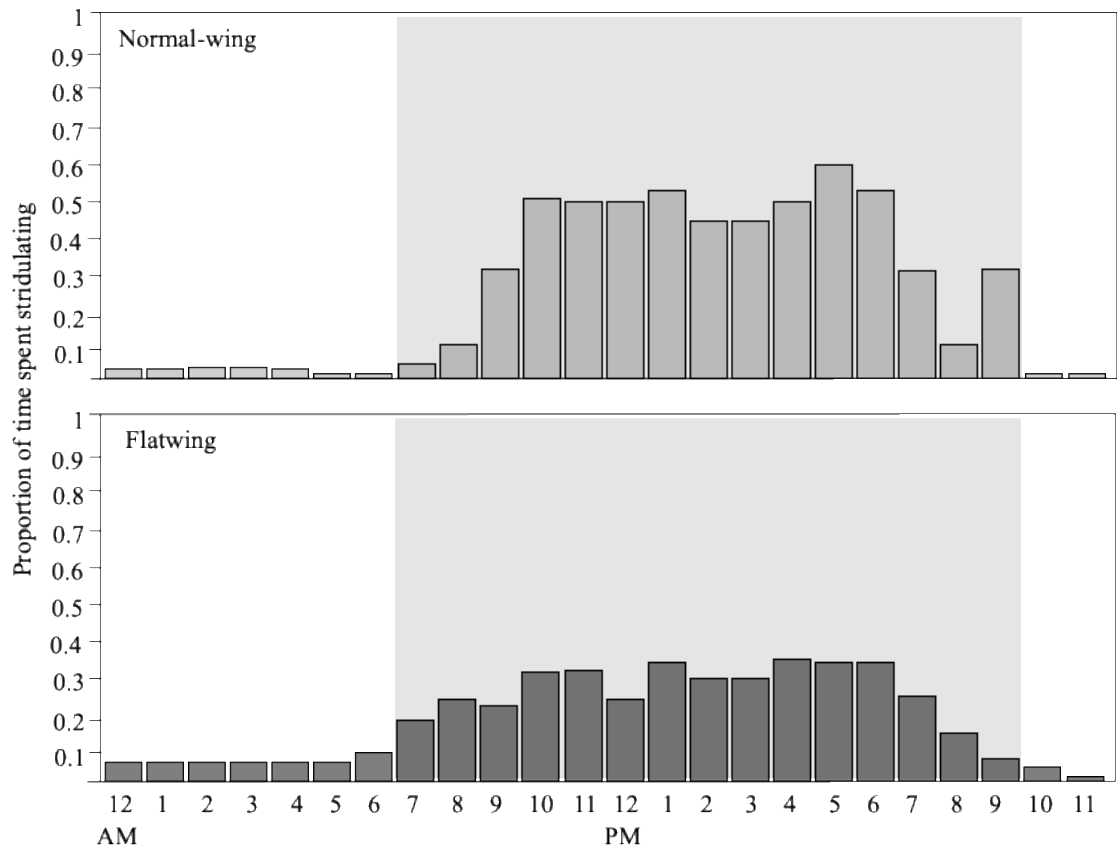
Source	No. of parameters	DF Num	DF Den	F-ratio	p-value
Morph	1	1	65	2.14	0.15
Photoperiod	1	1	67	102.24	<b>&lt;0.0001*</b>
Age	1	1	65	1.28	0.26
Morph* photoperiod	1	1	67	10.03	<b>0.0023*</b>
Morph* age	1	1	65	0.29	0.59

**Table 4.1** Summary of Generalized Linear Mixed Model (GLMM) fixed effects tests with response variable of proportion of time spent stridulating and fixed effects of: morph, photoperiod, and age. We used a random effect of individual and included interaction terms of morph by photoperiod and morph by age as fixed effects.

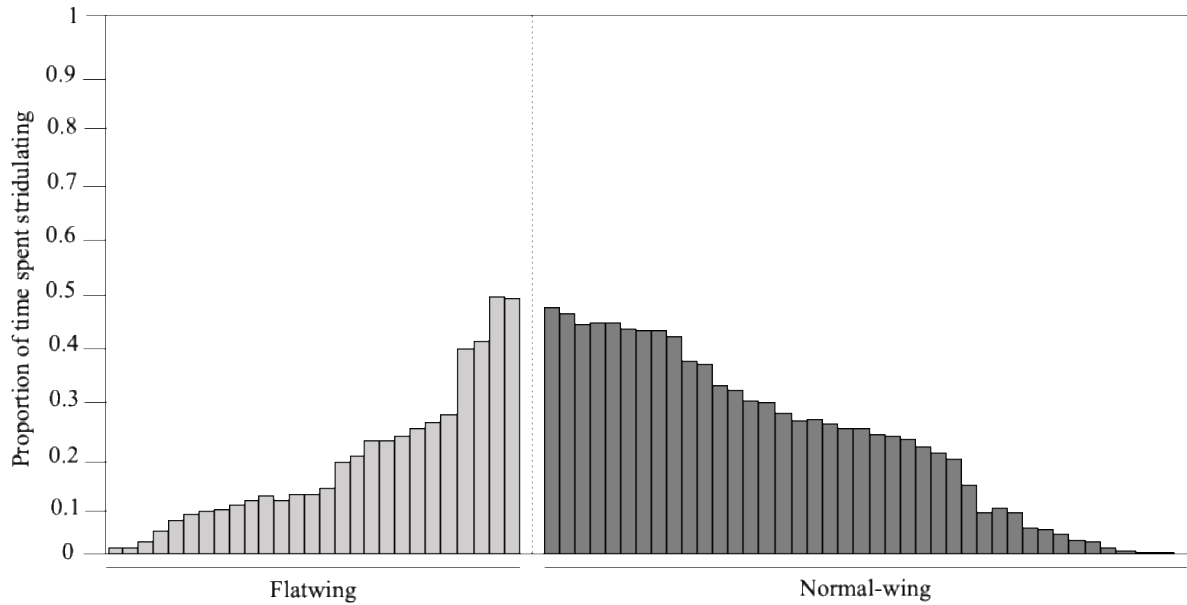
## Figures



**Figure 4.1** Proportion of 24-hour period spent stridulating by photoperiod and wing morph. Bars represent standard deviation of the mean. Males move their wings during the dark significantly more than during the light (GLMM, photoperiod:  $F_{1,67}=102.24$ ;  $p<0.0001^*$ ). There was a significant interaction between wing morph and photoperiod such that flatwings move their wings significantly more than normal-wings during the light hours (GLMM, morph\*photoperiod:  $F_{1,67} =10.03$ ;  $p=0.0023^*$ ). Flatwings ( $n=27$ ) stridulate during the dark 27.7% of the time and 6.78% of the time in the dark. Whereas normal-wings ( $n=41$ ) stridulate 43.2% of the time in the dark and 0.5% of the time in the light.



**Figure 4.2** Proportion of 24-hour period spent stridulating by time of day and wing morph. The bars within the dark square represent the dark hours of the 24-hour period. Flatwings stridulate more than normal-wings during the light hours (GLMM, morph\*photoperiod:  $F_{1,67} = 10.03$ ;  $p = 0.0023^*$ ).



**Figure 4.3** Proportion of 24-hour period spent stridulating averaged for each individual (N=68 males total). Each bar represents an individual with flatwings in light grey on the left and normal-wings in dark grey on the right. The variance between the two morphs is equal (ANOM–Levene’s test:  $F_{1, 67}=0.09$ ;  $p=0.77$ ).

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