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Those Who Wish to Sing Always Find a Song:
Call Loss and Reinvention in Hawaiian Crickets

by
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Part I – Introduction

One challenging aspect of studying evolution is that many of the changes we see in populations of organisms happen gradually over long periods of time. While there is a plethora of both observational and experimental studies that have documented changes in traits within populations over time (see University of California Museum of Paleontology, 2022), these changes are often subtle and require careful measurement to appreciate.

Dramatic, rapid changes do happen, though! One such change happened in the Polynesian field cricket, *Teleogryllus oceanicus*, a species that was introduced (i.e., is not native) to Hawaiian islands prior to the 1980s. In their native populations on islands elsewhere in the Pacific Ocean, males of these crickets, like those of many other cricket species, make calls and songs by stridulating (Box 1). Females, in turn, use those stridulations to locate males and make decisions about whether to mate with them.

Dr. Marlene Zuk, an evolutionary biologist and behavioral ecologist at the University of Minnesota, led a research team studying the introduced Hawaiian cricket populations on Kaua'i, one of the Hawaiian islands. While they observed crickets to be abundant and calling normally in 1993, her team documented a precipitous decline in their population over the coming decade (Figure 2, next page). In 2001, despite using the same sampling methods as in previous years, they encountered only one calling male during their field season, and the overall cricket population size had noticeably declined. When Dr. Zuk and her team returned in 2003, no males were heard calling.

Box 1 – How Crickets Generate Sounds

Crickets create their calls by stridulation: they rub specialized structures together on their wings at high frequencies to make loud, pulsing sounds. Microscopically, normal-calling males have a serrated structure on one wing called a file (attached along the curving vein at the tip of the blue arrow in Figure 1 below), which they rub on a scraper on the opposing wing to create a vibration (see images in Zuk et al., 2006). This vibration is then amplified by two speaker-like resonance structures found on the medial (rear-facing) forewing surface: a triangular harp and a round mirror (Figure 1). The result is a pulsing song that characterizes each cricket species.

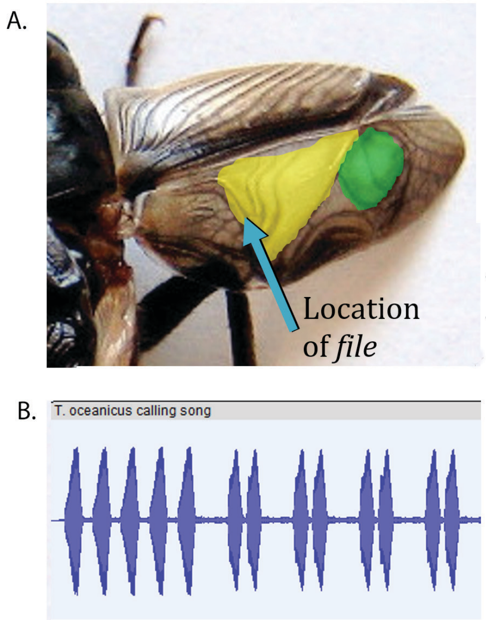

Figure 1. Panel A: the *harp* (yellow) and *mirror* (green), two wing structures that amplify vibrations created by the *file* (found along vein at tip of blue arrow) and *scraper*. Panel B: waveform showing pulses of one second of cricket song; you can listen to this cricket's song at <http://www.faculty.ucr.edu/~mzuk/T.%20oceanicus%20calling%20song.mp3>. (*Photo credit:* Robin Tinghitella.)

Nevertheless, they observed that the cricket population had rebounded! Males (and females) were abundant; they just were not stridulating (Zuk et al., 2006).

Curious about this change, Dr. Zuk and her team investigated the wings of these silent males and found dramatic changes. Figure 3 (next page) shows the forewings of typical (normal) males found elsewhere in the Pacific, and the newly observed males, dubbed flatwing males. In addition to the striking changes in wing venation pattern, microscopic investigation found that the file of flatwing males (see Figure 1) was approximately one fourth the length of that found on normal males (~500 μm vs. 2,000+ μm) and with a 60-degree shift in its orientation, such that it was oriented down the length of the wing rather than across it (Zuk et al., 2006).

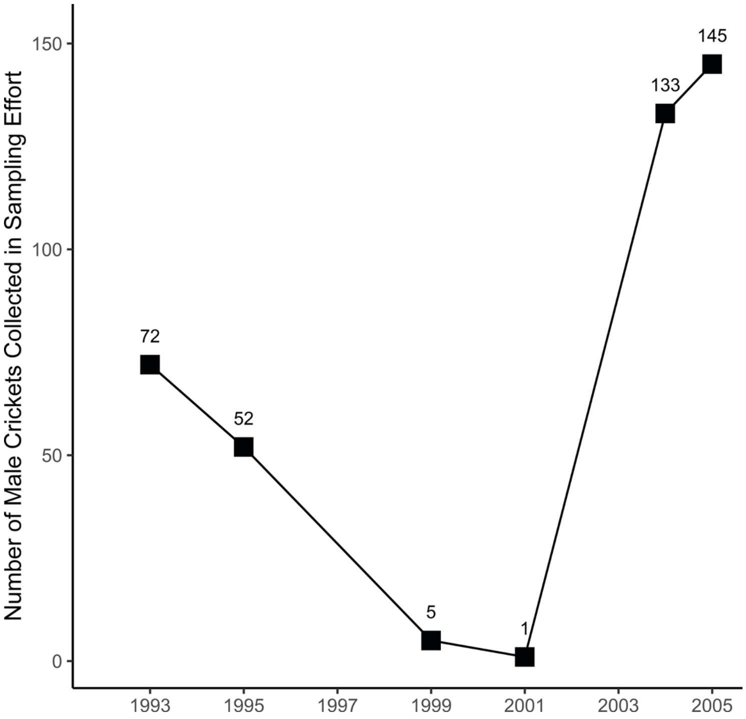

Figure 2. Number of male crickets collected using consistent sampling efforts, 1993–2005. Data from Zuk et al. (2006).

Questions

1. Examine Figure 3 carefully and compare the venation patterns on the forewings of the two individuals. Identify at least two ways that they differ. How might you expect these differences to affect the calls and songs of these individuals, based on Box 1? Remember: typical males make calls by rubbing specialized parts of their wings together.

2. What are some possible reasons for these differences? In other words, what could cause this population of crickets' wings to change so dramatically? There are many possibilities. Propose at least two ideas.

3. Evolution is often defined as a change in a population's genetic composition over time. Is this, therefore, an example of evolution? Or do we need additional information to decide? Why or why not?

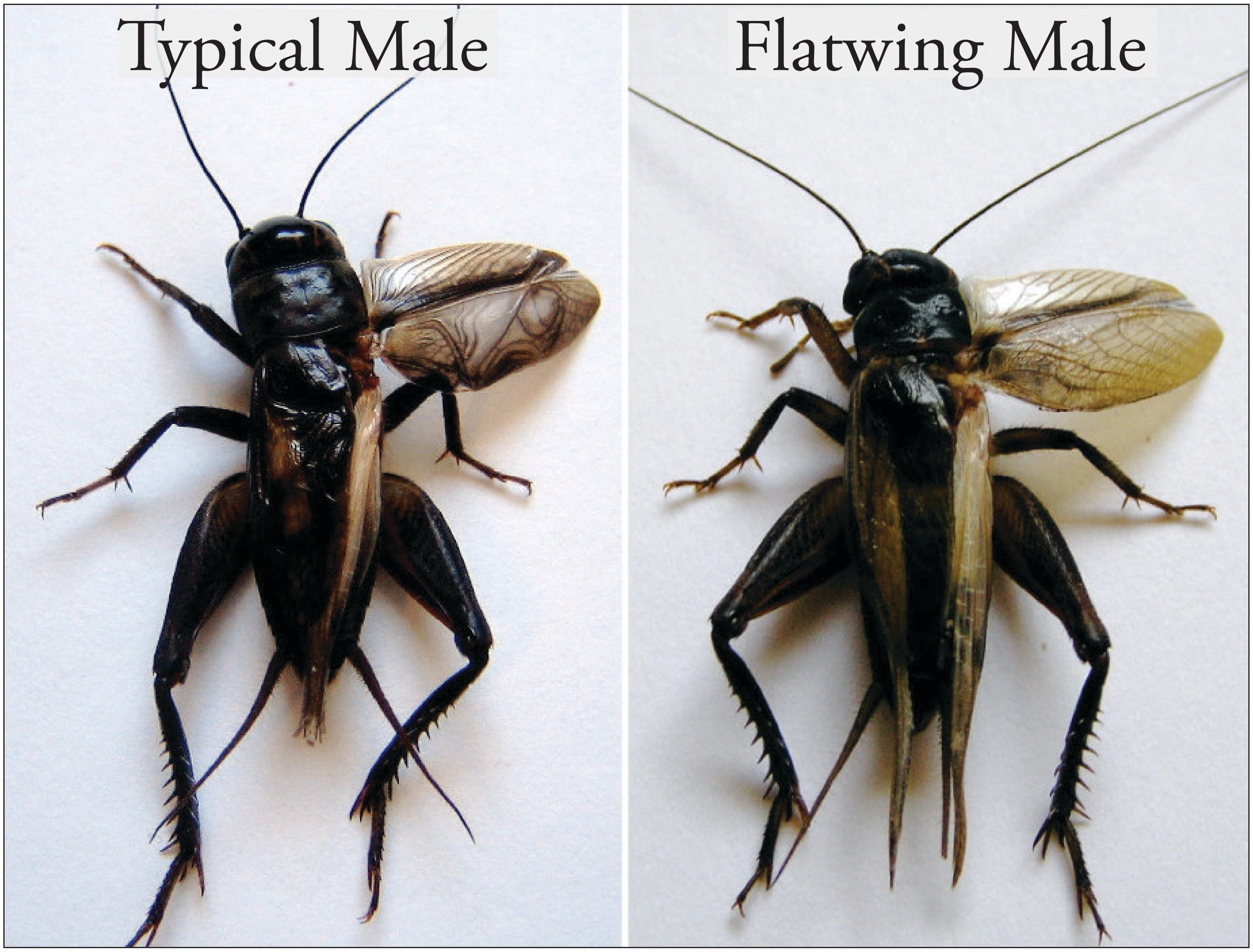

Figure 3. Normal males (from other islands in the pacific) capable of calling, and the “new” silent males, which Dr. Zuk’s lab named “flatwing” males. *Photo credit:* Robin Tinghitella.

Part II – Understanding the Change

To better understand what happened in this cricket population, we need to introduce another player in this system: the parasitoid fly, *Ormia ochracea* (Figure 4). These flies are native to North America, but they were also introduced into the Hawaiian islands, which is now the one location where their range overlaps with that of *T. oceanicus* crickets. Parasitoids reproduce by depositing their eggs or larvae onto a host species, which then feed upon the host. In this case, *O. ochracea* females deposit larvae onto crickets. The larvae then burrow into the cricket and consume it from the inside. About a week later, the fat larvae erupt from the husk of their now-dead cricket host, pupate, and then emerge as adult flies. These flies were particularly prolific on the island of Kaua'i, where more than 30% of singing males collected between 1992 and 1994 harbored fly larvae (Zuk et al., 1995).

Critically, *O. ochracea* females find crickets by their call. In fact, female flies of this species have specialized acoustic sensory organs (i.e., ears) that are most sensitive to frequencies ranging from 4 to 6 kHz (Robert et al., 1992; Box 2). This hearing range includes the 4.8 kHz songs generated by *T. oceanicus* males (Tinghitella et al., 2021)! *O. ochracea* female flies use their auditory organs to find hosts for their larvae, and readily approach stridulating male crickets. Not surprisingly, prior to the appearance of flatwing males, Dr. Zuk's team found that, while female crickets were occasionally parasitized, males were much more likely to be the subject of the flies' fatal interest (Zuk et al., 1993).

Question

4. Given the new information presented on this page, formulate an explanation for why the male crickets of this population lost their ability to generate calls. Try to be as specific as possible, describing how and why the population would change from a population of males capable of singing into this new population of flatwing, silent males.
   a. State your explanation:

   b. Explain your reasoning:

Figure 4. A gravid female *Ormia ochracea* fly resting on a fingernail. Photograph by Wikimedia Commons user Jpaur, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Ormia_ochracea_(gravid_female).jpg>.

Box 2 – Measuring Sound

Western musicians describe the different notes of a piano based on their pitch, which are each given different letter names: Middle-C, C#, D, and so on. The pitches that we perceive are the result of vibrating sound waves that travel through the air. Different pitches correspond to differences in how frequently the vibrations impact our ear drum. Low pitch notes have low frequency vibrations, whereas high pitch notes are the result of high frequency vibrations. We use the unit Hertz (Hz) to describe the number of cycles (vibrations) per second, and high frequency sounds are sometimes reported in kilohertz (1,000 Hz = 1 kHz).

A standard 88-key piano produces frequencies that range from 27.5 Hz (the lowest A key) to 4,186 Hz (the highest C key; 4.186 kHz). Middle-C has a frequency of 261 Hz. Most cricket sounds, by contrast, are about 5 kHz, which is approximately a D# in the next octave beyond a typical piano's range (Wikipedia, 2022b). Human ears are able to perceive sound ranging from approximately 20 Hz to 20 kHz (Wikipedia, 2022a).

Part III – Understanding Natural Selection

Your answer to Question 4 might have gone something like this: those crickets who were silent would not be attacked by the parasitoid, and consequently would have a survival advantage over stridulating male crickets. Therefore, because parasitism was fatal and the risk of parasitism was high, the silent flatwing crickets were more likely to survive (and reproduce, assuming they could still find and court a female) than the normal, calling crickets.

This explanation invokes natural selection, which is the mechanism originally proposed by Charles Darwin to cause evolution in populations. One framework for natural selection summarizes it as a process that is the inevitable consequence of three prerequisites:

If (1) a population has variation in a trait, (2) at least some of that variation in heritable, and (3) the variation affects the likelihood that individuals will reproduce, *then* natural selection will cause evolution within the population.

Therefore, natural selection will occur whenever our three prerequisites exist, i.e., whenever there is heritable variation in a trait that has reproductive consequences by affecting survival, attractiveness, competitiveness, fertility, etc. The most advantageous variations (i.e., the adaptations) should become more common in subsequent generations because those who have them will produce more offspring (i.e., higher fitness). Less advantageous variations should decline or disappear from the population because those who possess them will not reproduce as often.

Please apply these ideas about natural selection to our cricket example in the questions below.

Questions

5. Identify how the following two prerequisites for natural selection are satisfied in our example of Hawaiian *T. oceanicus* crickets, based on the information you have reviewed thus far:
   a. A population has variation in a trait:

   b. The variation affects the likelihood that individuals will reproduce:

6. Based on the framework for natural selection above, what would be expected to happen if there had never been variation in wing shape that influenced stridulation?

7. While we have not seen direct evidence of heritability thus far, what would happen if there was variation in stridulation, but it was not heritable?

8. In *T. oceanicus* cricket populations elsewhere in the Pacific Ocean, there are no *O. ochracea* flies. In those other populations, given that there are no parasitoids, how would the prerequisite that “variation affects the likelihood that individuals will reproduce” play out? In other words, would normal (stridulating) or flatwing (silent) males have higher fitness? Explain your answer.

9. In a different study, Dr. Zuk and colleagues investigated mating requirements in the female Hawaiian *T. oceanicus* cricket populations (Bailey et al., 2008). They found that the females did prefer normal, stridulating males compared to a silent male. However, unlike female crickets from other Pacific Island populations of this same species, who require close-range stridulation to mount males, female crickets from Kaua’i would still mate with a silent male about 50% of the time when housed together. Why is this information important to understanding how the flatwing trait spread through the population?

Part IV – Is It Heritable?

In Part III, we noted that one of the prerequisites for natural selection is that a trait must be heritable. The fact that we saw the spread of the flatwing variant through our cricket population is consistent with it being favored by selection, but a trait must be heritable to respond to selection.

Dr. Robin Tinghitella, then a graduate student in Dr. Marlene Zuk's lab and co-author on the original paper describing the flatwing crickets, set out to test whether there was a genetic basis of the flatwing variant (Tinghitella, 2008). First, she collected eggs from field-caught Kaua'i females. By this time, all males in the Kaua'i population had the flatwing traits. She reared crickets from those eggs for 12 generations under the same laboratory conditions that the lab previously had used to rear normal-winged males. In all cases, the male offspring in this study developed the flatwing trait; there were no normal-winged males or males with wings that were intermediate between normal and flatwings. This indicated that the trait was heritable and was not the result of environmental conditions: offspring reliably expressed the traits of their flatwing parents, lab conditions were the same as when the lab previously reared normal-winged males, and yet she only saw flatwing males among these offspring.

To probe deeper into the mechanism, Dr. Tinghitella established 28 mating pairs of crickets. Her parental males were silent, flatwing males collected from the Kaua'i cricket population. The parental females were from an archival, lab-reared Kaua'i stock of crickets that were collected several years before the appearance of the flatwing phenotype. Notably, all of the males from the lab stock had normal wings and no flatwing males had ever been seen in this lab lineage. She completed crosses of the flatwing males and normal females and then collected the first generation (F1) offspring. She then mated these F1 siblings together to produce a F2 generation.

Dr. Tinghitella hypothesized that the trait was controlled by a single genetic locus (Box 3), and she considered three possible inheritance patterns:

1. autosomal dominant (flatwing allele dominant to normal allele);
2. autosomal recessive (flatwing allele recessive to a dominant, normal allele); and
3. X-linked recessive (*note:* crickets use an XX/XO sex determination system; males have only one X chromosome, which they receive from their mother, and there is no Y-chromosome).

Let's generate predictions so that we can determine the inheritance pattern. For each scenario on the next page, use a Punnett square to calculate the expected percentage of male offspring with normal wings vs. flatwings (remember, the specialized wing structures are only found in males; female wings do not vary because their wings are used for flying, not stridulation). For simplicity, assume that the parental populations were true-breeding (i.e., homozygous), although this is an assumption we are making based on Dr. Tinghitella's experimental findings.

Box 3 – Genetic Locii

A *gene* is found at a specific location on a chromosome known as a *genetic locus*. Each gene may have several different variations known as *alleles*. Diploid organisms possess two copies of each chromosome, so individuals will usually have two alleles of each gene. An exception occurs with *X-linked traits*. In humans (and crickets), females have two X-chromosomes, but males possess only one. Therefore, females have two alleles for each X-linked gene, while males have only one allele because they have only one X-chromosome. The set of alleles that an individual possesses is their *genotype*, while the trait that they express is their *phenotype*. An individual's phenotype is determined by how their genotype interacts with an individual's environment.

Some traits are controlled by a single gene. In those cases, the alleles that an individual has at one locus will determine the phenotype. If an individual is *homozygous* (both alleles are the same) at the locus, their phenotype will correspond to that allele. When *heterozygous* (two different alleles at a locus), different possibilities exist. Some traits show a *dominant/recessive* pattern of inheritance. In these instances, heterozygous individuals will express the trait corresponding to the dominant allele. Other traits have *incomplete dominance* or *co-dominance* such that a heterozygote's phenotype will be either intermediate or a mixture of the phenotypes of homozygous individuals.

In many other instances, traits are controlled by numerous—often hundreds, if not more—genetic loci. In these cases, the impact of any one allele is often small, and an individual's phenotype will be determined by complex interactions between all of its alleles at each relevant locus. For more information, you may wish to review a biology textbook chapter on Mendelian genetics, like this one: <https://openstax.org/books/biology-2e/pages/12-2-characteristics-and-traits>.

Hypothesized Inheritance Pattern:	*Autosomal Dominant*	*Autosomal Recessive*	*X-Linked Recessive*
Parental Female Genotype:	*dd*	*RR*	$X^L X^L$
Parental Male Genotype:	*DD*	*rr*	X^l
Punnett Squares for Parental Cross			

Expected Results of Parental Cross

Ratio of Normal : Flatwing Males	:	:	:
% of Normal Wing Male Offspring	%	%	%
% of Flatwing Male Offspring	%	%	%

Now for the F1 Cross…

F1 Female Genotype:			
F1 Male Genotype:			
Punnett Squares for F1 Cross			

Expected Results of F1 Cross

Ratio of Normal : Flatwing Males	:	:	:
% of Normal Wing Male Offspring	%	%	%
% of Flatwing Male Offspring	%	%	%

Part V – The Data!

When Dr. Tinghitella completed her crosses, the data were as follows:

Male offspring in F1 generation:

Normal Males: 670 (99.9%)
Flatwing Males: 1 (0.01%)

Male offspring in F2 generation:

Normal Males: 171 (52%)
Flatwing Males: 160 (48%)

Question

10. Based on a comparison of these data to the predictions that you made in Part IV, which of the three hypothesized inheritance patterns was best supported? Explain your answer.

Part VI – Was That a … Purr?

Dr. Tinghitella continued to study these crickets after completing her doctoral work and starting her own lab at the University of Denver. While sampling crickets from the Hawaiian Island of Moloka'i in 2017, she and her team uncovered a population of *T. oceanicus* where males were making a new call reminiscent of a cat's purr (here an example at <https://tinghitellalab.weebly.com/uploads/2/8/3/2/28321271/mol211.1_co_09.02.2018__courtship_purring_.mp3>). The individual notes within the purring call were broad-band "clicks," compared to the discrete tones and harmonics of the ancestral (normal) call (Tinghitella et al., 2018). Other crickets on that same island were primarily flatwing males, which were now found in all Hawaiian islands (crickets seem to readily disperse between islands, perhaps aided by catching rides on boats or airplanes). Over the next several field seasons, Dr. Tinghitella and her students found increasingly large numbers of purring crickets in populations from O'ahu and Kaua'i (see Figure 5 for distribution in 2020). These were the same populations where the flatwing phenotype was ubiquitous just a few years before!

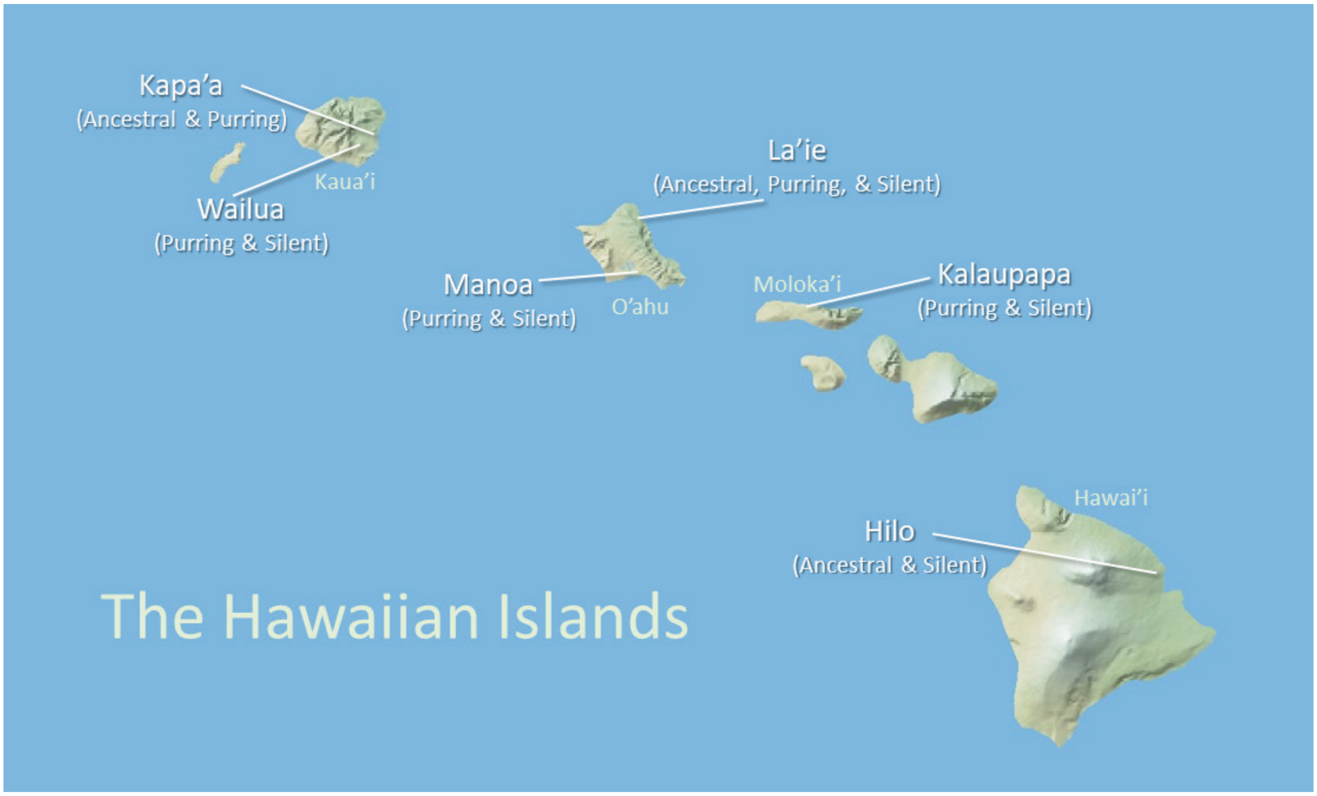

Figure 5. Six populations of *T. Oceanicus* crickets and relevant song type, as of 2020. Figure after Tinghitella et al. (2021). Public domain map courtesy of U.S. Geological Survey, National Geospatial Program, https://apps.nationalmap.gov/viewer/.

Question

11. The new purring trait appears to be spreading through Hawaiian populations of *T. oceanicus* crickets. Let's assume that this is happening because purring crickets are favored by natural selection over both normal and silent males.
    a. What fitness advantage might individual males with the new purring call have over silent, flatwing males?

    b. What fitness advantage might individual males with this new purring call have over males with the ancestral call?

To test the adaptive value of purring, Dr. Tinghitella and colleagues set up two experiments (Tinghitella et al., 2021). In the first experiment, they placed female crickets from the six Hawaiian populations (Figure 5) alone in sound-isolated arenas that contained a set of bluetooth speakers. Each speaker in the arena played one of three sounds: purring calls, ancestral calls, or white noise (a control). In the second experiment, they played the same recordings from speakers behind funnel traps designed to catch *O. ochracea* parasitoid flies at those same six field sites.

Question

12. Based on your answers to Question 11 above, make predictions. Phrase your predictions in terms of the specific data that you would expect to collect from each study.
    a. Based on your answer to Question 11(a), how should female crickets respond to the three sounds played from the speakers (ancestral, purring, or white noise)?

    b. Based on your answer to Question 11(b), how should *O. ochracea* parasitoid flies respond to the three sounds played from the speakers?

Part VII – Does the Purr Work?

The results were as follows:

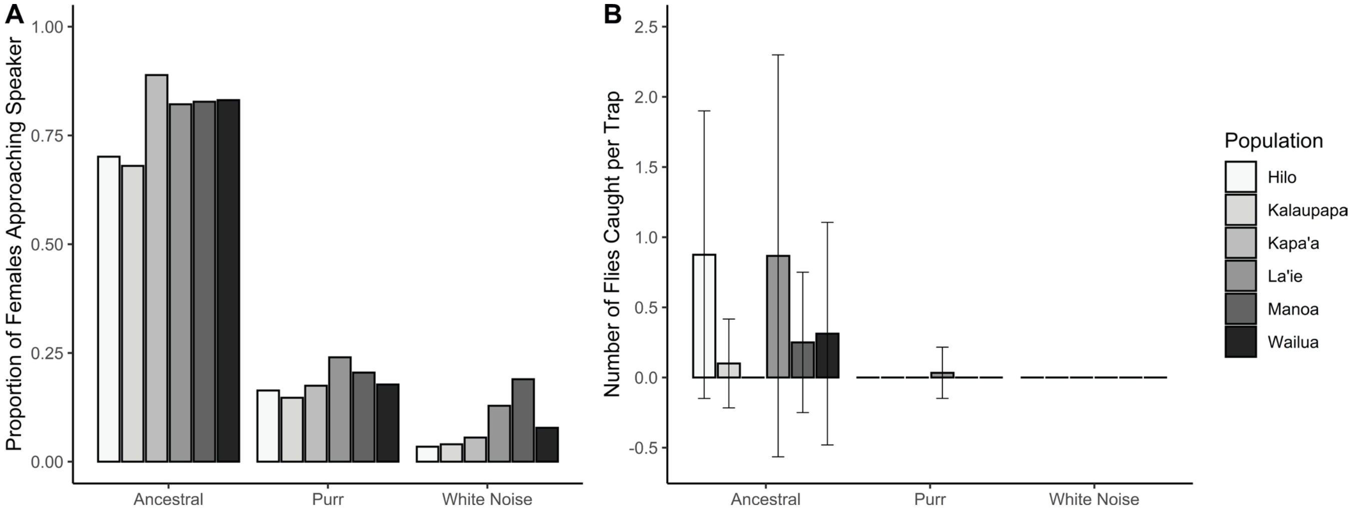

Figure 6. Female cricket (A) and parasitoid fly (B) responses to ancestral and purring songs. White noise was used as a control. Shaded bars indicate the field site where female crickets and flies were collected. Error bars in Panel B are standard deviation. Shaded bars represent the different Hawaiian cricket populations shown in Figure 5. After Tinghitella et al. (2021).

Panel A of Figure 6 shows the proportion of crickets who displayed phonotaxis (i.e., they approached the speaker playing the noise) for each sound tested. There were statistically significant differences in phonotaxis between each of the three sound types. Panel B of Figure 6 shows the average number of flies caught in traps from each field site. There was a significantly larger number of flies caught near the speakers playing ancestral calls compared to purring and white noise sounds. Only one fly was caught near a speaker playing the purring call, and no flies were caught next to a white noise speaker.

Questions

13. Let's evaluate these results in light of your answers to Questions 11 and 12.
    a. Regarding the experiment on female crickets:
        i. What advantage did you propose for purring males over silent, flatwing males (Question 11a)? *Note:* this proposed advantage is a hypothesis!

        ii. How did you predict cricket females would respond to each sound type (Question 12a)?

        iii. What do the data show about how female crickets responded to the three sounds?

iv. Do your predictions match the results? Why or why not? Explain your answer, and state whether these data support your hypothesized advantage of purring over flatwing males.

b. Regarding the experiment on *O. ochracea* flies:

i. What advantage did you propose for purring males over ancestral (normal) males (Question 11b)? *Note:* this proposed advantage is a hypothesis!

ii. How did you predict *O. ochracea* flies would respond to each sound type (Question 12b)?

iii. What do the data show about how *O. ochracea* flies responded to the three sounds?

iv. Do your predictions match the results? Why or why not? Explain your answer, and state whether these data support your hypothesized advantage of purring over ancestral males.

14. Speculate: based on these results, what calling behavior(s) do you predict to find in this cricket population 10 years from now? Explain your answer.

◆

Postscript

At the June 2021 Society for the Study of Evolution meetings, Jay Gallagher, one of Dr. Robin Tinghitella's graduate students, reported the discovery of yet another new T. oceanicus *cricket call, this time on the big island of Hawaii. This call sounds more like a rattle. These new males had different wing morphology from the purring males, and preliminary behavioral studies indicated that rattling was more attractive to female crickets than purring, while still offering protection from the flies! The story continues…*

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