Asymmetric Mating Preferences Accommodated the Rapid Evolutionary Loss of a Sexual Signal

ASYMMETRIC MATING PREFERENCES ACCOMMODATED THE RAPID EVOLUTIONARY LOSS OF A SEXUAL SIGNAL

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Rapid evolution has been well documented in naturally selected traits, but few examples exist for sexually selected traits, particularly sexual signals. This may in part be due to the complex set of behaviors associated with sexual signals. For a sexual signal to change, the change must be favorable for the signaler, but must also be accommodated by the receiver's perception and preferences. We investigated female accommodation of an extreme change in the sexual signal of Polynesian field crickets, *Teleogryllus oceanicus*. The cricket is native to Australia, widely distributed on Pacific Islands, and was recently introduced to Hawaii. Selective pressure by a deadly parasitoid fly favored a wing mutation in Hawaii (flatwing) that eliminates males' singing ability altogether. Despite conventional wisdom that females require males to produce a courtship song before mating, we show that females from ancestral, unparasitized Australian and Pacific Island populations as well as parasitized Hawaiian populations, will mate with silent flatwing males, suggesting this behavioral option predates the change in sexual signal. Furthermore, ancestral Australian females discriminate against flatwing males more severely than island females. We suggest island colonization favored females with relaxed mating requirements (Kaneshiro's effect) facilitating the rapid evolutionary loss of song in Hawaii.

KEY WORDS: Cricket song, female preference, island colonization, Kaneshiro's effect.

The movement of a species from location to location can be accompanied by progressive divergence of populations due to genetic drift and founder effects, and punctuated by more radical changes due to selection in new environments (MacArthur and Wilson 1967; Carson and Templeton 1984). These processes are particularly relevant to the spread of a species among islands. Both adaptive and nonadaptive forces shape the genetic structure and phenotypic divergence of island populations putting them in "one of the most favorable circumstances imaginable for rapid evolution" (MacArthur and Wilson 1967). More generally,

rapid evolution is frequently associated with introduction to novel habitats or local adaptation within heterogeneous environments (Reznick and Ghalambor 2001). This has been well established for naturally selected traits, but the manner in which sexual signals change in the context of founding events and subsequent selection is infrequently investigated (Zuk and Tinghitella 2008). Sexually selected traits should be amenable to rapid evolutionary change in recently colonized environments, just as naturally selected traits are, because a founding population's sexual signal may be well or poorly fit to a new environment (Yeh 2004). The genetic consequences of a founding event can influence male traits as well as female preferences (Meffert and Bryant 1991) and we expect transmission properties, competing signalers and eavesdropping natural enemies to influence signals in their new environments (Boake 2002).

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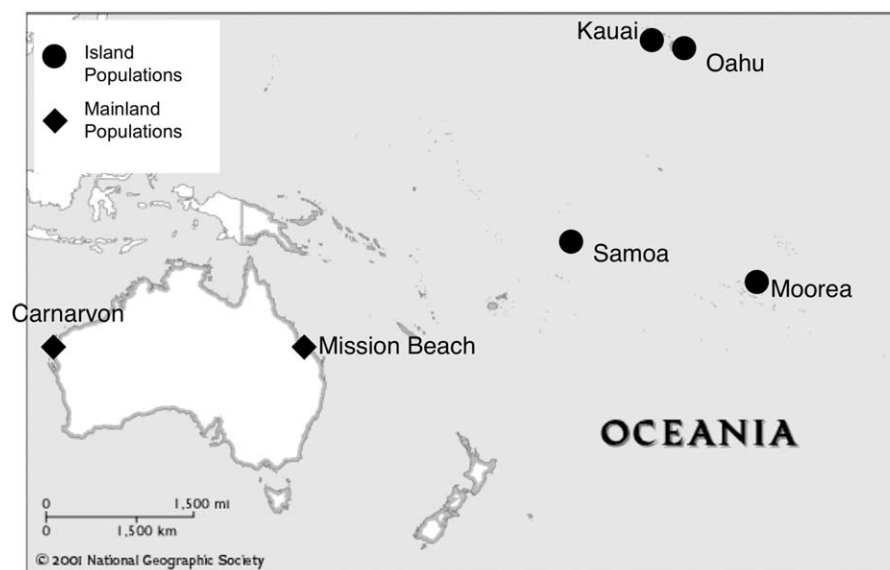
Figure 1. Polynesian field crickets, *Teleogryllus oceanicus*, have a broad distribution, spanning Northern and Eastern Australia, Oceania, and their introduced range in Hawaii. In Hawaii, the cricket co-occurs with the acoustically orienting parasitoid fly, *Ormia ochracea*. Six populations were used in this study of courtship interactions across the crickets' range and are indicated on the map. Two of these are from the crickets' native range of Australia, Carnarvon and Mission Beach (sometimes referred to as mainland populations), two of these are island populations where the parasitoid is not found, Samoa and Moorea, and the remaining two are island populations from Hawaii where the cricket and fly coexist, Oahu and Kauai. More then 90% of males in the Kauai population have the mutant wing morphology, flatwing.

Given the myriad forces exerted on sexually selected traits, surprisingly few examples of rapid evolutionary change in sexual signals have been found in natural populations (Svensson and Gosden 2007). This may in part be due to the complex set of behaviors associated with sexual signals. Signals and associated behaviors form a "functional complex" (Palmer et al. 2007); for a sexual signal to change, not only must the change be favorable for the signaler, it must also be accommodated by the receiver's perception and preferences. If for one trait to change, another must change first, the evolution of such complexes is constrained. This is a familiar circumstance for many life-history characteristics (Roff 1992). However, constraint is not the only possible outcome of the close relationship between signaling and behavior. Behavior is extremely variable both within and among individuals, and this property can accommodate sexual signal evolution if preexisting behaviors or plasticity therein allow for a given change in a signal. This could instead speed rates of evolution (Zuk and Tinghitella 2008). There has been much recent interest in the role of phenotypic plasticity in either constraining (by shielding genotypes from selection) or facilitating contemporary evolution (by generating novel opportunities for selection to act) (Yeh and Price 2004; Ghalambor et al. 2007). Behavioral variation may play a similar role. Here, we investigate whether preexisting variation in behavior was in place prior to the loss of the sexual signal of male Polynesian field crickets, *Teleogryllus oceanicus*, such

that female responses to male courtship were "preadapted" to accommodate the change in male signaling.

The population history of *T. oceanicus* is characterized by multiple founding events and strong divergent selection on sexual signaling across their range. The cricket is native to Australia and widely distributed on Pacific Islands, having been introduced to Hawaii sometime before 1877 (Otte and Alexander 1983; Kevan 1990, Fig. 1). Neutral genetic variation in microsatellites is consistent with a general pattern of spread from Australia east to Hawaii; we find there is a strong pattern of isolation by distance and a reduction in allelic diversity in eastern islands relative to mainland western populations (R. M. Tinghitella and M. Zuk, unpubl. data). In colonizing the Pacific, the crickets likely underwent multiple founding events as they moved from west to east. Only in Hawaii (Lehmann 2003), *T. oceanicus* encounters a deadly parasitoid fly (*Ormia ochracea*) that is attracted to the song males make to locate females (Cade 1975; Zuk et al. 1993). In one study, over 30% of males dissected harbored parasitoid larvae (Zuk et al. 1993), and the fly is responsible for changes in the crickets' sexual signal and associated behavior (Zuk et al. 1995, 1998, 2001; Lewkiewicz and Zuk 2004). Most recently, on the Hawaiian Island of Kauai, selective pressure by the fly favored a wing mutation (flatwing) that eliminates males' singing ability altogether (Zuk et al. 2006). Silent flatwing males lack the structures on the wing (file, scraper and resonating structures)

necessary to produce song. The mutation is controlled by a single sex-linked locus (Tinghitella 2008) and spread to >90% of males in fewer than 20 generations because it protects them from the parasitoid (Zuk et al. 2006).

Field cricket females ordinarily locate stationary signaling males by phonotaxis to their calling song, a long-distance conspicuous signal (Alexander 1962). A lower intensity short-distance courtship song is produced by males in mating interactions, and females normally mate only after first hearing this song (Balakrishnan and Pollack 1996). Thus, flatwing male *T. oceanicus* have two novel obstacles to overcome: (1) attracting females, and (2) convincing them to mate without song. Silent flatwing males appear to overcome this complication by behaving as satellites, approaching calling males and attempting to mate with females attracted to them (Zuk et al. 2006). These males avoid the risk of calling themselves and there is apparently little risk associated with pursuing a satellite-like behavior in this population. In a field study, only one of 121 flatwing males was parasitized (Zuk et al. 2006), a rate lower than that experienced by females in this population (who also spend time near calling males) before the mutation spread (~7%; Zuk et al. 1995).

What remains to be seen is how females respond to flatwings during courtship. During courtship, the male contacts the female's body with his antennae, vibrates his antennae, adopts a singing posture, and begins producing courtship song (Burk 1983). Mating proceeds when the female mounts the male in his singing posture. Although 80–90% of females mount males after the initiation of courtship song (Balakrishnan and Pollack 1996), when males have been artificially muted, they frequently cannot elicit mounting (Crankshaw 1979; Adamo and Hoy 1994; Libersat et al. 1994). Despite conventional wisdom that females should require males to produce a courtship song, the persistent population of primarily flat-winged *T. oceanicus* on Kauai suggests this difficulty has been overcome.

Because secondary sexual traits are intricately involved in mate location and courtship interactions, when they are lost or change rapidly, we are challenged to explain why this is so. Many sexually selected traits are known to arise and be maintained by female choice, so for a male trait to change or be lost, the mating advantages that males gain by having a given trait must be over ridden by some other force such as random or environmental effects (for instance strong natural selection against the male trait), or female preferences must be reduced in some way (Wiens 2001). Some, but not all, evidence from groups in which male traits have been lost supports the idea that relaxed female preferences "allow" rapid changes or loss of male traits. For instance, in swordtails (Morris 1998), Anas spp. ducks (Omlund 1996), and guppies (Endler and Houde 1995) female preferences are less strong in groups in which male sexual traits have been lost. In the case of guppies, strong predation pressure on colorful males and females that associate with them favored the reduction of female preferences, but there is insufficient evidence to show that the loss of preference led to the reduction in male coloration (Houde and Endler 1990). In a second example in which males of two closely related species of swordtails (*Xiphophorus nigrensis* and *X. pygmaeus*) lost their vertical bars, female preferences favor the bars in one species but discriminate against them in the other (Morris 1998). Also in swordtails (*X. cortezi*), a polymorphism in female preferences for males with or without vertical bars may maintain both male types (Morris et al. 2003). One disadvantage of several of these studies is that they are necessarily performed long after the loss of the male trait. We have the advantage of investigating the evolution of male traits and female preferences in a dynamic system in which the loss of a male secondary sexual trait was directly observed, recently, and over a short period of time (Zuk et al. 2006).

Additionally, we can ask questions about the cause of variation in female preferences. One suggestion that has been controversial (Giddings and Templeton 1983) is Kaneshiro's hypothesis (1976, 1980, 1989) that the initial stages of island colonization, when population size is very small, impose strong selection for females who are less discriminating in mate choice. Kaneshiro (1980) proposed that extremely choosy females would be unlikely to mate under these circumstances. This, he argued, would relax selection on sexual signals, favoring divergence and premating reproductive isolation. Kaneshiro's hypothesis predicts a characteristic mating asymmetry whereby ancestral females discriminate against derived males (who may lack elements of the courtship repertoire), but derived females discriminate less strongly between ancestral and derived males. In this way, historical events (colonization history) and subsequent selection interact to influence the evolution of sexual signals and reproductive isolation.

Kaneshiro's verbal model has been criticized on the grounds that it lacks general applicability (Giddings and Templeton 1983). The model requires a demographic history in which species (or populations) can be thought of as ancestral and derived, and where derived groups are isolated after a founding event and population flush. It has also been criticized because the process and outcome Kaneshiro predicts is just one of a variety of equally likely outcomes of island colonization (Arnold et al. 1996). We agree. Nevertheless, the loss of male traits and relaxation of female preferences is one mechanism that may facilitate the evolution of signaling traits following colonization, relaxing the relationship between male signals and associated behavior and reducing constraint in those systems.

We suggest populations of *T. oceanicus* are a good candidate for exhibiting Kaneshiro's effect. The cricket originated in Australia and spread east through the Pacific (Otte and Alexander 1983; Otte 1994), setting up a system of ancestral and derived populations that includes several extremely isolated island

populations in which there is presumably little gene flow among islands. There is an established genetic basis for both male song production and female preferences for song (Bentley and Hoy 1972; Simmons 2004), and cricket mating systems are characterized by female choice (Alexander 1962). Although originally proposed for Hawaiian *Drosophila* species, mating asymmetry has been identified in organisms including European *Drosophila* (Sperlich 1964), sticklebacks (McPhail 1969), and native Hawaiian crickets (Shaw and Lugo 2001) and has been extended to include within species variation in female preferences (McPhail 1969; Bicudo 1978).

We investigated the response of females from six populations across the range of *T. oceanicus* to flatwing males (who clearly lack courtship elements) and ask whether a reduction in female preferences evolutionarily precedes the loss of male singing ability, or, is instead associated with or even due to the loss of singing ability. In a previous study, Bailey et al. (2008) found that females from the flatwing Kauai population and a population from the same location, but established in the laboratory prior to the flatwing mutation, were willing to accept the courtship advances of males that had been artificially silenced in approximately 40% of no-choice mating trials. Their observation is consistent with the hypothesis that mating behavior of females accommodated this rapid morphological change. It does not, however, distinguish between the possibilities that local selection on Kauai crickets favored the acceptance of silent males (as in guppies where selection to avoid costly matings favors relaxed female preferences for bright males in areas of high predation; Houde and Endler 1990) or that preferences vary across the crickets' range, potentially as a byproduct of island colonization. We address two questions. First, do females from across the crickets' range accept the courtship advances of silent flatwing males? Second, do island females have relaxed mating requirements relative to females from mainland populations? If females from across Australia and the Pacific sometimes mount flatwing males, this suggests variation in female mating requirements preadapted females to accept the courtship advances of silent flatwing males in Hawaii and that the relaxation in female preferences is not due to the loss of the male trait (as one might predict if there was selection to reduce female mating requirements following the loss of the male trait). Furthermore, if island females have consistently relaxed mating requirements relative to mainland females, this suggests that the nonadaptive effects of colonization play a role in generating the variation in female preferences that facilitated this loss.

Methods

POPULATION ORIGINS AND ANIMAL MAINTENANCE

We chose six populations for this study: Kauai and Oahu (two parasitized island populations), Moorea and Samoa (two unpar-

Table 1. Population origins and supplementation. Unless otherwise noted, laboratory populations were supplemented with the offspring of field-caught females approximately once per year to avoid the potential impacts of inbreeding.

Population	Year established	Supplementation
Kauai	2003	Yearly
Oahu	1991	Yearly
Moorea	2003	None
Samoa	2006	Yearly
Mission Beach	2006	Yearly
Carnarvon	1996	< Yearly

asitized island populations), and Mission Beach and Carnarvon (two unparasitized mainland Australian populations; see Fig. 1). All populations were maintained in the laboratory and consisted of at least 100 breeding adults at any given time. Table 1 indicates the dates on which these populations were established. Unless otherwise noted, the populations were supplemented with the offspring of field-caught females approximately yearly to minimize the effects of inbreeding. *Teleogryllus oceanicus* have three to four generations per year. The only population used that contained flatwing males was Kauai. Although there are a few remaining normal-winged (calling) males on Kauai, none were used in this experiment. Juvenile crickets were maintained in temperature-controlled growth chambers set at 28°C on a 12:12 light:dark schedule. Prior to the penultimate molt, juvenile females were removed and placed in single-sex 15 L containers, approximately 30 females per container with ad libitum access to food (Fluker's cricket chow), water, and egg carton for cover. We surveyed for eclosed females at least three times per week. Upon eclosion to adulthood, females were moved to smaller (1 L) containers (< 10 females per container) and treated identically except that their food was replaced with Purina rabbit chow.

MATING TREATMENT

Because mating history has been demonstrated to affect female mating behavior in field crickets (with virgin females exhibiting greater phonotaxis; Lickman et al. 1998), we ensured that all females mated exactly two times prior to their use in a "final" courtship trial. After females attained an age of at least five days post-eclosion they were allowed an opportunity (up to once per day) to mate with a male from their own population. These matings were conducted in a temperature-controlled room set at 25°C under dim lights. A mating opportunity consisted of placing the female with a male (pulled at random from a larger stock population) in a small plastic container (156 mL) for up to 2 h and allowing her to mate with him. For each female, we noted the dates of her mating opportunities and scored each opportunity as successful if a spermatophore was transferred during the encounter. Only after a female had mated twice was she used

in the final courtship trials. Females who did not complete the mating treatment (two successful matings in six opportunities) were removed from the experiment. Approximately 3% of females did not mate in at least two of six opportunities.

CROSS-POPULATION COURTSHIP TRIALS

To examine female mating decisions across the range of *T. oceanicus*, we used "no choice" courtship trials, in which one male and one female were offered an opportunity to mate, because the more common "male choice" or "female choice" designs are confounded by male–male or female–female interactions respectively, and complications due to differing mating propensities of different populations (Arnold et al. 1996). Our design is adapted from Shaw and Lugo (2001), who investigated sexual isolation and mating asymmetry in endemic Hawaiian *Laupala* crickets. No-choice courtship trials for all possible combinations of the six aforementioned populations were performed for a total of 36 types of courtship pairings. We performed >25 individual trials of each courtship type for a total of 1081 final courtship trials. Courtship trials between females and males from their own population (homotypic combinations) were used to establish the "normal" mating rate, to which heterotypic combinations would later be compared. The number of successes (mounting by female within 5 min of the onset of courtship song) and failures (no mounting) of homotypic and heterotypic combinations were recorded to examine the relative degree and direction of sexual isolation between populations.

A no-choice courtship trial consisted of placing a female of known mating history in a 1 L container (6 inch × 8 inch in size) and then placing the container into one of four acoustically isolated compartments (such that up to four courtship trials could be run simultaneously). A 1 L container size was chosen because it was large enough that females could escape the courtship advances of a male if she chose. A male (pulled at random from the larger stock population) was then introduced through a hole in the clear lid on the 1 L container. We noted the latency between the introduction and onset of courtship song by the male, mounting speed (the latency between the onset of courtship song and mounting by the female), and mounting outcome. Trials ended 5 min after the male began producing courtship song. Courtship containers were wiped down with a 10% bleach solution between trials. Because flatwing males do not produce song, but do retain the stridulatory behavior associated with calling, we were able to watch for wing movement to determine when courtship began. A flatwing was considered to have begun courtship when he first vibrated his wings and assumed a calling posture. We defined mounting as presence of the female on top of the male for at least 2 sec. We did not allow the cross-population mating trials to proceed to spermatophore transfer so that females could be retained to propagate breeding stocks. Males were given only 5 min

in which to begin courtship song. If they did not, they were replaced. Females who were not courted by four consecutive males were removed from the study. This was extremely rare.

We staged a total of 1119 final no-choice courtship trials. No animals were reused in the final courtship trials (those trials not intended to ensure females had mating experience). In 38 of these, the trial was discarded because the female was not courted by four consecutive males. These trials were distributed throughout the 36 different courtship types with the following frequencies (female population listed first): once in each of the following combinations, Kauai × Oahu, Kauai × Kauai, Kauai × Moorea, Oahu × Samoa, Mission Beach × Samoa, Mission Beach × Oahu, Samoa × Oahu; twice in each of the following combinations, Moorea × Kauai, Mission Beach × Mission Beach, Oahu × Samoa, Moorea × Mission Beach, Samoa × Samoa, Moorea × Samoa, Mission Beach × Moorea, Kauai × Mission Beach; three times in Moorea × Oahu trials; and, four times in each of the following, Moorea × Moorea, Oahu × Mission Beach, Carnarvon × Moorea. The distribution of these failed courtship encounters among homotypic and heterotypic pairings and across the broad geographic distribution of the populations suggests the failure of males to court is due to oddities associated with certain interactions, rather than a consistent failure to recognize females from certain populations, or premating reproductive isolation. The removal of these 38 trials left 1081 final no-choice courtship interactions.

Females in final courtship trials had attained a mean age of 15.24 ± 0.22 days posteclosion (mean $\pm$ 1 SEM). Adult females were at least seven days old, but not more than 30 days old. The mean age of females who mounted (15.33 ± 0.25 days) and did not mount (14.99 ± 0.45 days) courting males did not differ ($t = 0.629$, $P = 0.5291$).

STATISTICAL ANALYSIS

Following Shaw and Lugo (2001), we performed a heterogeneity *G*-test (Sokal and Rohlf 1995, p. 715–724) to determine whether mounting frequencies among the 36 courtship types were homogeneous and to identify the sources of any heterogeneity. We were interested in significant differences between populations in the propensity to mount within populations (estimated by the frequency of homotypic mountings), and significant differences between populations in the frequency of heterotypic mountings. To determine which population combinations were responsible for heterogeneity, we partitioned the total *G* value into separate *G*-tests representing individual degrees of freedom from each of the 36 courtship pairings. We corrected for multiple comparisons using a Bonferroni correction. An additional heterogeneity *G*-test was used to determine whether mounting frequencies with flatwing males differed when they were paired with island or mainland females, or when they were paired with Hawaiian females versus non-Hawaiian females.

Table 2. Summary of results for heterogeneity *G* tests of mounting rate. *G*-values are calculated for an expected mounting frequency of 0.810, the average homotypic mating frequency. *significant after Bonferroni correction. Superscripts refer to mainland (m), island (i), and Hawaiian (h) populations, respectively.

Male population	Female population	Proportion mounting	df	*G*
Carnarvon	Carnarvon[m]	0.840	1	0.172
Carnarvon	Mission Beach[m]	0.900	1	1.907
Carnarvon	Samoa[i]	0.833	1	0.128
Carnarvon	Moorea[i]	0.750	1	0.649
Carnarvon	Oahu[i,h]	0.867	1	0.726
Carnarvon	Kauai[i,h]	0.500	1	14.321*
Mission Beach	Carnarvon[m]	1.000	1	11.935*
Mission Beach	Mission Beach[m]	0.867	1	0.726
Mission Beach	Samoa[i]	0.934	1	3.841
Mission Beach	Moorea[i]	0.767	1	0.315
Mission Beach	Oahu[i,h]	0.867	1	0.726
Mission Beach	Kauai[i,h]	0.833	1	0.128
Samoa	Carnarvon[m]	0.750	1	0.568
Samoa	Mission Beach[m]	0.800	1	0.012
Samoa	Samoa[i]	0.900	1	1.907
Samoa	Moorea[i]	0.800	1	0.012
Samoa	Oahu[i,h]	0.645	1	4.513
Samoa	Kauai[i,h]	0.800	1	0.012
Moorea	Carnarvon[m]	0.800	1	0.012
Moorea	Mission Beach[m]	0.800	1	0.012
Moorea	Samoa[i]	0.700	1	2.009
Moorea	Moorea[i]	0.867	1	0.726
Moorea	Oahu[i,h]	0.867	1	0.726
Moorea	Kauai[i,h]	0.833	1	0.128
Oahu	Carnarvon[m]	0.880	1	0.935
Oahu	Mission Beach[m]	0.900	1	1.907
Oahu	Samoa[i]	0.767	1	0.315
Oahu	Moorea[i]	0.900	1	1.907
Oahu	Oahu[i,h]	0.875	1	1.026
Oahu	Kauai[i,h]	0.865	1	0.839
Kauai	Carnarvon[m]	0.094	1	77.098*
Kauai	Mission Beach[m]	0.200	1	51.76*
Kauai	Samoa[i]	0.267	1	41.239*
Kauai	Moorea[i]	0.414	1	21.898*
Kauai	Oahu[i,h]	0.406	1	25.032*
Kauai	Kauai[i,h]	0.500	1	14.321*
Total G			36	284.488*
Pooled G		0.736	35	32.99*
Heterogeneity G			1	251.498*

We tested for differences in the latency of males to court females in no-choice trials using a two-way analysis of variance (ANOVA) with male population, female population, and their interaction as factors. Finally, taking a shorter latency to mount to be evidence of greater preference, we tested for the independent effects of male population and female population, as well as their interaction on time to mate using a two-way ANOVA.

Results

Mounting rates ranged from a low of 9.4% in courtship encounters between Carnarvon females and flatwing Kauai males to a high of 100% in courtship encounters between Carnarvon females and Mission Beach males (Table 2, Fig. 2). The overall frequency of successful mountings was 0.715; in homotypic pairings this
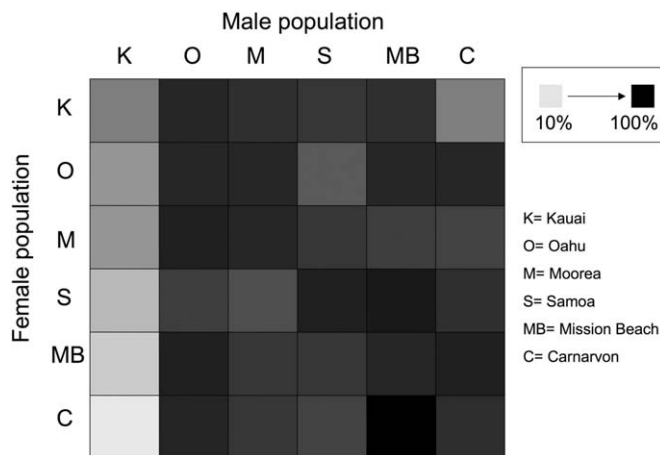
Figure 2. Mounting frequencies in the 36 staged courtship combinations. The degree of transparency indicates the frequency with which females mounted males provided to them. Lighter shades are indicative of lower mounting rates and darker shades indicate higher degrees of acceptance by females.

Table 3. Summary of results for heterogeneity *G* test of mounting rates with flatwing Kauai males only. *G*-values are calculated for an expected mounting frequency of 0.50 (the within population mating rate for Kauai). *indicates terms that were significant after Bonferroni correction. Superscripts refer to mainland (m), island (i), and Hawaiian (h) populations, respectively.

Male population	Female population	df	*G*	Proportion mounting
Kauai	Carnarvon[m]	1	24.449*	0.094
Kauai	Mission Beach[m]	1	11.565*	0.200
Kauai	Samoa[i]	1	6.794	0.267
Kauai	Moorea[i]	1	0.866	0.414
Kauai	Oahu[i,h]	1	1.132	0.406
Kauai	Kauai[i,h]	1	0	0.500
Total *G*		6	44.806*	
Pooled *G*		5	26.671*	
Heterogeneity *G*		1	18.135*	

increased to 0.81 (ranging from a low of 0.5 in Kauai flatwing pairs to a high of 0.9 in Samoa pairs; Table 2). *G*-values were therefore calculated with an expected mounting frequency of 0.81. The results for the overall *G*-test of heterogeneity can be found in Table 2, and mounting frequencies for all 36 courtship types are represented graphically in Figure 2. Mounting rates across the 36 pairings were significantly heterogeneous ($G = 284.488$, $P < 0.0001$), prompting us to make further comparisons to identify the source of heterogeneity. After correction for multiple comparisons, eight courtship pairings deviated significantly. Six of these were the pairings of females from across the crickets' range with flatwing Kauai males. All pairings with Kauai flatwing males were significantly less likely to result in mounting by females than pairings with males from any other population tested (all $P < 0.0001$ after correction for multiple comparisons). The other two heterogeneous mating combinations were the pairings of Kauai females and Carnarvon males, in which only 50% of trials ended in mounting, and the combination of Carnarvon females and Mission Beach males in which all trials ended in mounting by the female. Despite the isolation observed between Kauai females and Carnarvon males, Carnarvon males did not appear to discriminate against Kauai females in courtship initiation. They initiated courtship song with Kauai females as quickly as they did with females from the other five populations ($F_{5,171} = 1.148$, $P = 0.3371$).

We used an additional heterogeneity *G*-test to examine the relationships between females from the six geographically isolated populations and flatwing males from Kauai in particular. An expected mounting rate of 0.50 was used for comparison (the within population mounting rate of Kauai females with flatwing Kauai males). Mounting rates across the six populations were signifi-

cantly heterogeneous ($G = 18.135$, $P < 0.05$), and after correction for multiple comparisons, pairings of Kauai males with Australian females from Carnarvon and Mission Beach were revealed to be significant sources of heterogeneity ($G = 24.449$, $P < 0.05$ and $G = 11.565$, $P < 0.05$ respectively; Table 3). Females from Carnarvon and Mission Beach were significantly less likely to mate with flatwing Kauai males. This is consistent with Kaneshiro's mating asymmetry: ancestral (mainland) females discriminate against derived flatwing males more strongly than do more derived females from isolated island populations.

Males differed overall in their latencies to begin producing courtship song ($F_{35,1045} = 4.67$, $P < 0.0001$). A two-way ANOVA found a main effect of male population on latency to court ($P < 0.0001$, Fig. 4A), and a main effect of female population on the latency of males to court ($P < 0.0001$, Fig. 4B). There was a significant interaction between male population and female population ($P < 0.0001$, Table 4) such that the speed with which a male began courtship singing depended on his population and that of the female he encountered.

Among those females who mounted males in the no-choice trials, mounting speeds varied as well ($F_{35,759} = 1.74$, $P = 0.006$, Table 4, Fig. 4C, D). A second two-way analysis of variance (ANOVA) found a main effect of male population on female mounting speed ($P = 0.0011$, Fig. 4C), but no main effect of female population on mounting speed ($P = 0.5134$, Fig. 4D). There was a significant interaction of male population and female population ($P = 0.0411$) suggesting the speed with which females from different populations mounted depended on the male's source population. In other words, taking faster mounting response to be evidence of a greater preference, female preferences were not consistent across populations.

Table 4. Summary of ANOVA results for latency of males to begin courtship and female mounting speed. Analysis of variance of the latency of males to begin courtship song and female mounting speed are presented separately. Male population and female population are included as main effects, and the male population × female population interaction term indicates whether male response or female responses vary when presented with courtship partners from different populations. *Indicates terms that were significant.

|	df	Sum of squares	F	P
Latency to court				
Female population	5	344417.18	13.27	<0.0001*
Male population	5	201362.27	7.76	<0.0001*
Female pop. × Male pop.	25	331233.20	2.55	<0.0001*
Error	1045			
Total	1080			
Latency to mount				
Female population	5	21078.18	0.85	0.5134
Male population	5	101639.44	4.11	0.0011*
Female pop. × Male pop.	25	192615.66	1.56	0.0411*
Error	759			
Total	794			

Figure 3. Acceptance of flatwing male *T. oceanicus* by females from across the crickets' range. Open bars represent females from ancestral mainland Australia populations and filled bars represent females from derived island populations. *indicates population pairings that were significant sources of heterogeneity following Bonferroni correction for multiple comparisons. Pairings with Samoa females were a source of heterogeneity before, but not after Bonferroni correction.

In additional analyses, we asked whether latency to court explains variance in mounting success to assess whether male assessment or recognition of females contributed to the pattern of mounting we observed. In a regression analysis, latency to court did not explain variance in mating speed in this dataset ($r^2 = 0.000172$, $F_{5,793} = 0.1363$, $P = 0.7121$). Females who were courted after longer periods of time did not also then take longer to mount males. In addition, for each male population, we asked whether males courted females from certain populations with different speeds (taking a shorter latency to court to be evidence of greater preference or recognition). Six one-way ANOVAs (corrected for multiple comparisons) with female population as a factor and latency to court as the response variable revealed that, in general, males did not prefer to court females from certain populations (or alternatively did not take longer to recognize females from some populations as potential mates). Mission Beach and Oahu males did, however, respond to females from different populations with faster or slower courtship ($F_{5,172} = 8.0143$, $P < 0.006$ and $F_{5,178} = 11.0651$, $P < 0.006$ respectively). Males from the other four populations did not vary the speed with which they initiated courtship depending on the female with whom they interacted. Both Mission Beach and Oahu males began courting females from Carnarvon more quickly than females from the other five populations and began courting females from Mission Beach more slowly than those from the other populations.

Discussion

Behavioral variation in female mating preferences was in place in populations ancestral to those where singing ability has been lost. As expected, we found little variation in the propensity of females from the six tested populations to mount males from any of the five populations where song is still produced, and mounting rates were high. However, in pairings with flatwing males, the mounting rates ranged from a low of 9.4% (in the western Australian Carnarvon population) to a high of 50% (in the Kauai flatwing population). Despite conventional wisdom that crickets require courtship song before mounting, at least some females from all populations relax this requirement (Fig. 2), but flatwing males are accepted at lower rates than normal-winged males. Females from across the crickets' broad geographic range retain a preference for calling males, but importantly, at least some females from all populations occasionally accept males who do not produce the sexual signal. Overall, females mounted flatwing males in 31.3% of our no-choice trials, consistent with the >40% mounting rate Bailey et al. (2008) found for Kauai males who had been artificially silenced. That at least some females from all populations accepted the advances of silent flatwing males suggests there is likely genetic variation underlying this trait that existed in the ancestral populations, crossed the ocean with the migrants, and occurs more frequently in island populations.

Ancestral mainland Australian females discriminated more strongly against flatwing males than did derived females from

C= Carnarvon
M= Moorea
MB= Mission Beach
O= Oahu
S= Samoa
K= Kauai
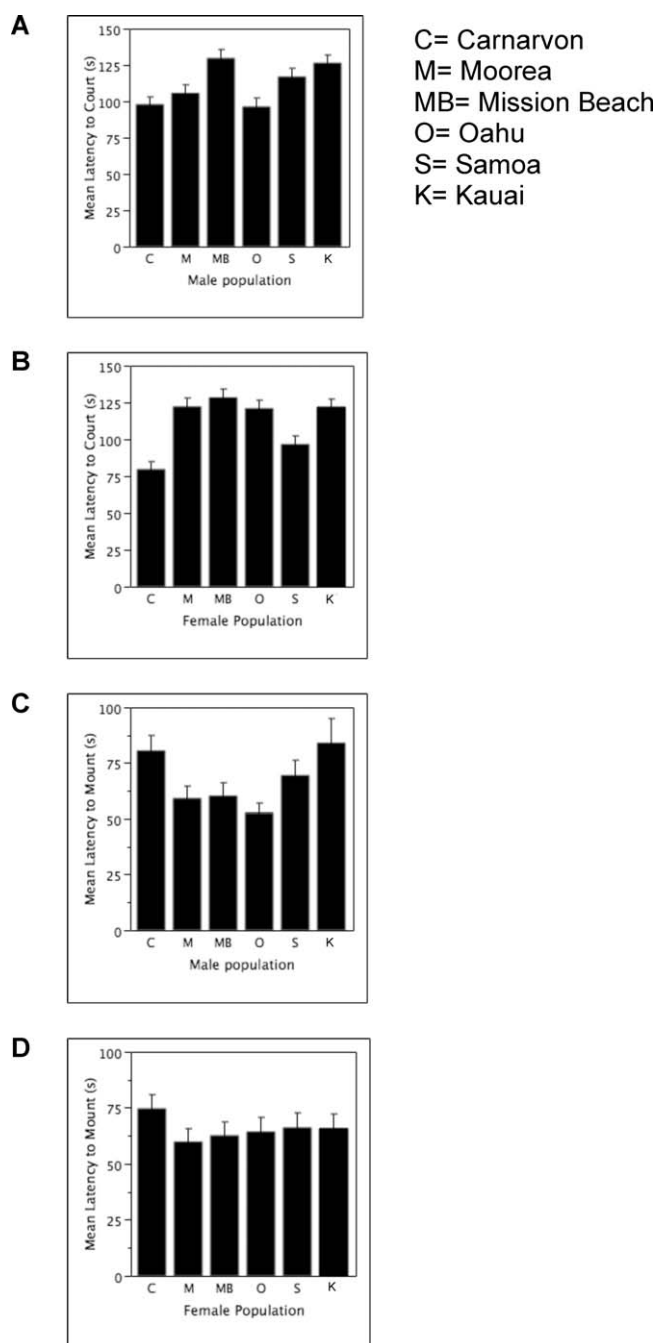
Figure 4. We found overall variation in the speed with which males began courtship singing and the speed with which females mounted for mating. Bars indicate ± 1 SE.

island populations (Fig. 3, Table 3), with only 9.4% of Carnarvon females and 20% of Mission Beach females mounting flatwing males. Derived island females maintained a preference for ancestral males and distinguished less strongly between mainland and island males. The asymmetric pattern of mating preferences is not consistent with the hypothesis that Hawaiian females accept flatwing males for mating due to selection to avoid becoming parasitized themselves. This is because females from other islands,

such as Moorea, were equally likely to mount flatwing males (Fig. 3). The pattern is consistent with Kaneshiro's hypothesis (1976, 1980, 1989) that founder effects upon island colonization select for females with relaxed mating requirements (because extremely choosy females are less likely to mate in small founding populations). We interpret these results cautiously, however, because many courtship traits may vary between Kauai and the other populations tested (for instance cuticular hydrocarbons and tactile signals). The definitive test of females' response to the nonsinging phenotype per se would require testing females from each of the populations with males from their own population, some of whom were experimentally silenced and others who were not.

The mating preferences of females from Australia and Samoa are less distinct from one another than Australian behavior is from the other three island populations tested (Fig. 3). We suspect this is because Samoa and Australia are less isolated geographically from one another and perhaps experience ongoing gene flow. Furthermore, if island colonization favors females who are less choosy, this effect may be magnified by repeated colonization events. Assuming the crickets moved through the Pacific from west to east, colonizing islands along the way (which is likely given their limited flight ability and short generation times; Zuk et al. 1998), Samoan *T. oceanicus* would have experienced fewer bottlenecking events in their population history than other island populations we investigated. We know, for instance, that genetic differentiation among populations is affected more strongly by sequential founder events than single colonization events (Clegg et al. 2002). Detailed analyses of neutral genetic variation are currently underway to elucidate the relationships among *T. oceanicus* populations, definitively establish their route of colonization in the Pacific, detect the genetic signature of bottlenecking events, and identify the presence or absence of gene flow among populations.

The relatively high (50%) rate at which Kauai females mounted flatwing males probably facilitated the spread of a wing mutation that now eliminates the sexual signal of more than 90% of males in their population. Variation in mating requirements precedes the loss of the sexual signal evolutionarily and seems to have generated an opportunity for selection to favor the loss of a sexual signal, which presumably would otherwise be maladaptive. Wiens (2001) suggested that for a sexually selected trait to be lost, the sexual selection pressures that maintain the trait must first be relaxed. Further empirical and theoretical studies are needed to demonstrate whether it is typically the case that female mating requirements are relaxed prior to the loss of male signaling characteristics, or as a result of the loss of male signals (Wiens 2001). Some evidence in diverse taxa supports this contention, but it has been difficult to demonstrate the order of events because the loss of courtship elements is so rarely observed directly. This study presents one case, one population, with a loss of male calling ability where there was also preexisting variation in female

mating requirements and strong natural selection against calling males. Can we conclude that relaxation in female preferences facilitated the loss of male singing ability based on observations of one population? Our observations on a second Hawaiian Island, Oahu, where there is also relaxation of female preferences (Fig. 2) and selection against calling males (Zuk et al. 1993) suggests that this pattern may be more general. Over 12–16 generations (since September 2004), we observed an increase in frequency of the flatwing trait from negligible to >50% of males on Oahu (R. M. Tinghitella and M. Zuk, unpubl. data). In this study, females from Oahu accepted flatwing males in >40% of courtship trials (Table 2). We continue to monitor the spread of flatwing morphology on Oahu.

In addition to the six courtship pairings that involved Kauai flatwing males, one other population pairing was a significant source of heterogeneity for mounting rate. This was the combination of Kauai females and Carnarvon males in which only 50% of trials ended in mounting by the female. These two are the most geographically isolated of the populations we tested. We expected Kauai females to be less choosy than others in general, but clearly there is some unexplained reproductive isolation between Kauai females and Carnarvon males. This suggests there are mechanisms of divergence at work in this system that we have not identified. One possibility is that Carnarvon males did not recognize Kauai females as mates and altered their courtship behavior. No other female populations discriminated disproportionately against Carnarvon females (Fig. 2, Table 2). Furthermore, the low mating rate observed between Kauai females and Carnarvon males appears to be driven primarily by females, rather than males. Carnarvon males did not discriminate against Kauai females or prefer females from other populations to those from Kauai when courting; rather they initiated courtship song with Kauai females equally as quickly as they did those from all other locations. The nature of female preferences may also play a role here. Females may be more responsive as a result of island colonization (responding to a broader range of males or more strongly to preferred males), but still retain a range of acceptability outside of which some Carnarvon males fall.

In addition to identifying geographic variation in the response of females to males from different populations (mount vs. no mount), we found variation in male responsiveness to females (latency to court) and the speed with which females responded to male courtship (latency to mount) (Fig. 4A–D, Table 4). The latency of males to begin producing courtship song in staged no-choice courtship trials was dependent on the interaction of male and female population (Table 4). We take this to mean males from different populations vary in intrinsic motivation for courting and that this also depends on contextual cues from the female with whom he interacted.

The latency of females to mount males who began courting was also dependent on the interaction of male population and female population (Table 4). The significant interaction effect in analyses of both latency to court and latency to mount begs the question, is latency to mount a reflection of latency to court? If latency to court explains variance in mating success, male assessment or recognition of the female as a potential mate may be an alternative explanation for the variation in mounting rates that we observed across the six populations. We found no evidence that variation in male courtship contributed to variation in the speed with which females mounted males. Females who were courted after longer periods of time did not take longer to mount males.

We suspect that the latency to court more accurately reflects when the male and female first contacted one another, rather than male assessment. Both Mission Beach and Oahu males began courting females from Carnarvon more quickly than females from the other five populations and began courting females from Mission Beach more slowly than those from the other populations. This may reflect male preferences for Carnarvon females and for other females over Mission Beach females, although it would be unusual to find that males from Mission Beach preferred all other females to those from their own population. Males from the other four populations did not vary the speed with which they initiated courtship depending on the female with whom they interacted. Additionally, field crickets are classically considered a female choice system. Unlike Mormon crickets, for instance, they provide no nuptial gift or large spermatophores to females during mating. For this reason, it is unlikely there would be selection to wait to mate if there is a female at hand. The patterns revealed in latency to court (that Mission Beach and Oahu males court Carnarvon females quickly, but Mission Beach females slowly) were not reflective of the mounting rates we found in these courtship combinations. More than 87% of these mating interactions resulted in mounting by the female, and none were significant sources of heterogeneity in mounting rate. We suggest that variation in courtship speed may instead reflect differential activity levels of females, such that Carnarvon and Mission Beach females are more and less active (respectively) resulting in earlier and later encounters with males. The mating containers used were large enough to allow females to avoid nonpreferred males, and individuals frequently explored the mating container before encountering one another opportunistically. Finally, the failure of males to begin courting at all was very low (38/1119 trials) suggesting that male recognition of females was not a problem.

It is interesting to note that mating asymmetry is recognizable in this dataset only when we consider the categorical loss of a sexual signal. There is considerable variation in the sexual signals of *T. oceanicus* from across Australia and Oceania (calling song, Zuk et al. 2001). Yet, females from the six populations we sampled only have asymmetric mating preferences with regard to males

from Kauai. Australian females do not discriminate against Oahu males, for instance, more strongly than do females from island populations (see Fig. 2). Perhaps the courtship song (to which females were exposed in this study) does not vary geographically as much as calling song or perhaps females do not exert preferences for small variations in the song. Alternatively, mating asymmetry may be best observed when entire courtship elements are absent (as in the song of male *T. oceanicus* or the red coloration lacking in some populations of sticklebacks studied by McPhail (1969)). This is precisely the situation to which Kaneshiro (1976, 1980, 1989) referred. Shaw and Lugo (2001), however, demonstrated a similar effect among pairs of Hawaiian *Laupala* species that differ only in the pulse rates of their mating songs, not the presence or absence of a sexual signal.

Here, we show that variation in female mating preferences existed in ancestral populations and preceded the rapid evolutionary loss of singing ability in an island population of field crickets. This variation in mating requirements may have been amplified in derived populations owing to the interaction of the nonadaptive consequences of island colonization and subsequent selection. So, we can link the influence of nonadaptive events (island colonization) and selection (by natural enemies in the introduced range) on sexual signal evolution. This type of behavioral compensation may be an important mechanism for invading populations when rapid evolutionary changes in sexual signaling, or other types of traits, occur soon after colonization (Baldwin 1896; Holway and Suarez 1999; Yeh and Price 2004).

ACKNOWLEDGMENTS

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