Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation

Kerry L. Shaw[1] and Sky C. Lesnick

Department of Biology, University of Maryland, College Park, MD 20742

Edited by May R. Berenbaum, University of Illinois at Urbana-Champaign, Urbana, IL, and approved April 10, 2009 (received for review January 9, 2009)

The genetic coupling hypothesis of signal-preference evolution, whereby the same genes control male signal and female preference for that signal, was first inspired by the evolution of cricket acoustic communication nearly 50 years ago. To examine this hypothesis, we compared the genomic location of quantitative trait loci (QTL) underlying male song and female acoustic preference variation in the Hawaiian cricket genus *Laupala*. We document a QTL underlying female acoustic preference variation between 2 closely related species (*Laupala kohalensis* and *Laupala paranigra*). This preference QTL colocalizes with a song QTL identified previously, providing compelling evidence for a genomic linkage of the genes underlying these traits. We show that both song and preference QTL make small to moderate contributions to the behavioral difference between species, suggesting that divergence in mating behavior among *Laupala* species is due to the fixation of many genes of minor effect. The diversity of acoustic signaling systems in crickets exemplifies the evolution of elaborate male displays by sexual selection through female choice. Our data reveal genetic conditions that would enable functional coordination between song and acoustic preference divergence during speciation, resulting in a behaviorally coupled mode of signal-preference evolution. Interestingly, *Laupala* exhibits one of the fastest rates of speciation in animals, concomitant with equally rapid evolution in sexual signaling behaviors. Genomic linkage may facilitate rapid speciation by contributing to genetic correlations between sexual signaling behaviors that eventually cause sexual isolation between diverging populations.

Laupala | sexual isolation | signal | speciation

The evolutionary and genetic mechanisms causing variation in sexual signaling systems have challenged biologists for decades (1–6). On the one hand, sexual communication between males and females should be evolutionarily constrained given that signals must match preferences to remain functional. If signals or preferences outside the natural distribution of variation cause their bearers to suffer a mating disadvantage they should be under stabilizing sexual selection (3). This stabilizing selection likely explains the frequent observation of behavioral coupling within species. On the other hand, even the most closely related species are typically differentiated in components of the sexual signaling system (7–8), demonstrating frequent divergence in mating signals and responses. Because sexual signaling systems are nearly always distinct among species, and their differences can cause sexual isolation among species, the evolution of sexual signaling is likely a powerful force driving speciation (9, 10). Despite a long history of interest in speciation and sexual selection, the evolutionary processes and the genetic conditions by which sexual signals diverge among species remain poorly understood.

Theory suggests several mechanisms can cause the coevolution of signals and preferences, and much empirical evidence supports a role for mate choice in divergent evolution of sexual signaling (11). Sexual selection models often assume free recombination between signal and preference loci, yet predict that a genetic correlation between signal and preference behaviors will arise within a population because of assortative mating. Such genetic correlations can accentuate the evolutionary effects of directional sexual selection when signal and preference distributions are mismatched within a population (11). Two recent studies, one in the olfactory realm (12) and the other in the visual realm (13), have suggested that physical linkage or pleiotropy might bolster behavioral coupling of sexual signaling in the face of divergent evolution, an idea with a long history (1, 6) but little empirical support. Furthermore, recent modeling suggests that physical linkage can promote a genetic correlation between signal and preference and thereby enhance coevolution in sexual signaling systems (14).

In crickets (family Gryllidae), males sing with specialized structures on the forewings to produce a calling song, and females respond to the calling song of potential mates by walking toward the acoustic source. These conspicuous sexual communication behaviors first inspired the genetic coupling hypothesis as a possible mechanism to explain widespread and coordinated evolution of male signal and female preference (1). The distinctiveness of male song (and female acoustic preference when it has been tested) (15) has proven highly useful in resolving species-level relationships, and thousands of species initiate courtship using such cues (e.g., see refs. 16 and 17). Research on male song and female acoustic preference in some species has shown a genetic correlation between song and preference (18), a pattern that could simply be because of assortative mating and sexual selection, or due to physical linkage of these traits (or both). Behavioral coupling in parental species and behavioral intermediacy of songs and preferences in first generation hybrids have yielded genetic insights (2) but cannot address the question of physical linkage (3). However, in a segregating hybrid population (e.g., an F_2 intercross or backcross) the role for physical linkage or pleiotropy as a potential cause of genetic correlations can be tested.

Species of the endemic Hawaiian cricket genus *Laupala* are morphologically and ecologically cryptic, and they exhibit the fastest rate of speciation known among invertebrates (19). We tested the hypothesis of genetic linkage between signal and preference in this system, where both male calling songs and female acoustic preferences have diverged repeatedly (17, 20–22) and contribute to sexual isolation (15) among closely related species. *Laupala* species with distinct songs can be hybridized, enabling studies of interspecific genetic architecture (23). Here, we employ a quantitative trait locus (QTL) approach to map female acoustic preference loci that differ between the closely related *Laupala paranigra* and *Laupala kohalensis*, and we combine these results with previous QTL work on male calling song differences between these same species (23). We demon-

Author contributions: K.L.S. designed research; K.L.S. and S.C.L. performed research; K.L.S. contributed new reagents/analytic tools; K.L.S. and S.C.L. analyzed data; and K.L.S. and S.C.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be sent at the present address: Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853. E-mail: kls4@cornell.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0900229106/DCSupplemental.

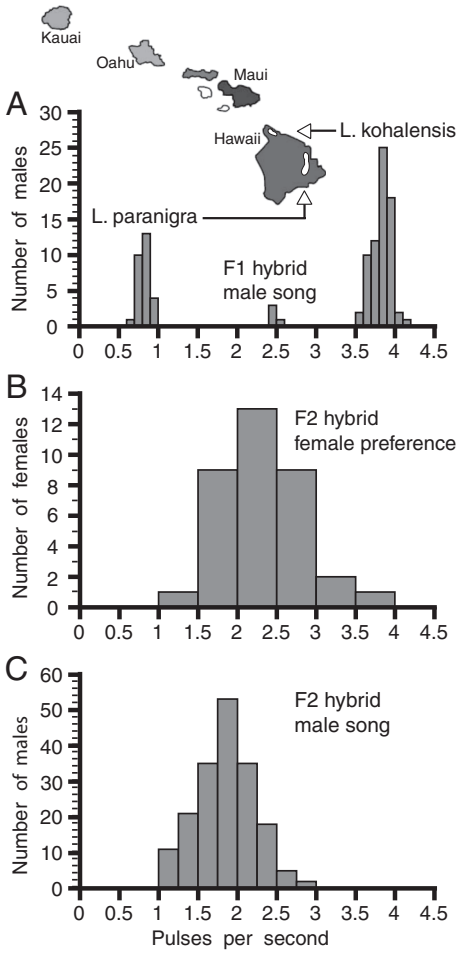

Fig. 1. Geographic range and male pulse rate variation of *L. paranigra*, *L. kohalensis*, and F_1 hybrids (*A*) (23); F_2 hybrid female pulse rate preference variation (*B*); and F_2 hybrid male pulse rate variation (*C*) (23).

strate colocalization of song and preference QTL, an observation consistent with the genetic coupling model of acoustic communication and sexual signaling system evolution.

Results

Phenotyping. Female *L. kohalensis* and *L. paranigra* show preference for male songs with species-characteristic pulse rates (21). Here, we estimated pulse-rate preferences for F_2 hybrid females ($n = 35$) from a cross between these species (23), representing >800 phonotaxis trials. Preferences segregated between the pulse-rate range of the 2 parental species and were normally distributed (preference mean ± SD = 2.28 ± 0.54; Shapiro–Wilk: $W = 0.949$, $P = 0.108$) (Fig. 1). Preferences could not be estimated for an additional 10 females (22%), which tended to be unresponsive in acoustic trials.

The male songs of *Laupala* species differ primarily in pulse rate (17, 20). Male *L. kohalensis* and *L. paranigra* songs have pulse rates of 3.72 ± 0.128 pulses per second (pps) and 0.71 ± 0.079 pps, respectively (Fig. 1*A*) (23). Previously, the pulse rates of 190 F_2 males from this cross were reported (23) (Fig. 1*C*), showing a normal distribution between the pulse rates of the 2 parental species (F_2 pulse rate mean ± SD = 1.86 ± 0.36; Shapiro–Wilk: $W = 0.994$, $P = 0.636$) (23).

Genotyping and Linkage Mapping. Linkage maps for this study included 183 amplified fragment length polymorphism (AFLP) markers (91 and 92 markers in the *L. paranigra* and *L. kohalensis* maps, respectively). Based on segregation patterns from 181 F_2 males and 47 F_2 females, 8 linkage groups for the *L. paranigra* map (Lp) (Fig. 2*A*) and 8 linkage groups for the *L. kohalensis* map (Lk) (Fig. 2*B*) were estimated (23), equal to the number predicted by cytological analysis (24). Most AFLP markers are dominant, but inclusion of several codominant AFLP markers allowed alignment of the Lp and Lk maps (Fig. S1) (23). Map size estimates were 613.1 and 476.6 cM, and average marker spacings were 7.5 and 5.8 cM for Lp and Lk, respectively (23).

QTL Analysis. We identified a single acoustic preference QTL on Linkage Group 1 in both Lp and Lk maps (Fig. 2), based on those females whose phenotypic and genotypic data were available ($n = 26$). QTL analyses for preference and song were performed by using interval mapping (IM) and composite interval mapping (CIM) with 5% experiment-wide error thresholds (Fig. 2) (25). The logarithm of odds (LOD) peak on Lp6b exceeded the 10% threshold and was used as a covariate in the Lp CIM analysis (Fig. 2*A*). The orthologous region to Lp6b has not yet been identified in Lk (Fig. S1) (23), thus preference analysis of Lk was only performed with IM (Fig. 2*B*). The estimated map positions (location of maximum LOD score; Table 1) for the preference QTL on Lp1 and Lk1 coincide with estimated map positions for song QTL from previous analyses (23). The marker closest to the estimated QTL (PcaacA5B12) is identical in all analyses and the 1.5 LOD support intervals (26) coincide between song and preference in both Lp and Lk (Fig. 2).

The additive effect of the preference QTL was significantly different from 0 (*t* test, $P < 0.05$), and no dominance deviation was detected. In the Lp and Lk analyses, the estimated effect sizes for the preference QTL were 0.45 and 0.44 pps, corresponding to 15% and 14.6% of the pulse rate difference between *L. paranigra* and *L. kohalensis*, and explaining 47.1% and 47.7% of the F_2 preference variance, respectively (Table 1).

Discussion

Sexual signals and their responses are conspicuous, often ornate, features of animal behavior. Sexual signaling systems in general, and song and acoustic preferences in particular, play a critical role in both intraspecific pair formation and sexual isolation between species (10). A genetic understanding of how such systems diverge may yield insights into how evolution within species gives rise to new species. It has been known for decades that the songs of male crickets attract females and that closely related species differ in these acoustic behaviors, prompting hypotheses about how songs and preferences remain behaviorally coupled in the face of divergent evolution (1–6). In *Laupala*, evidence supports the hypothesis that song and preference are integral to pair formation (27) and that differences in pulse rates of closely related species elicit discriminating female preferences, thus supporting a role for acoustic behavior in barriers to gene flow (15). *Laupala* shows the fastest rate of speciation yet measured in invertebrates (19), and a consistent pattern of song differences between close relatives supports the hypothesis that song evolution plays a causative role in speciation in this group.

In this study we found that a QTL for pulse rate of the male calling song colocalizes with a QTL for female pulse rate preference. As such, this study is significant in reporting a physical linkage of genes underlying male and female acoustical communication behaviors. Only 2 previous studies of the genetic basis of behavioral coupling provide evidence for physical linkage or pleiotropy of sexual traits, each representing a different sensory modality. In the fruit fly *Drosophila melanogaster*, mutations at the *desat1* gene modify the cuticular hydrocarbon quantities of both males and females, and male sexual response to them (12). Likewise, in *Heliconius* butterflies, male recognition of conspecific wing color pattern mapped to the same region as female (and male) wing color pattern (13). Both of these

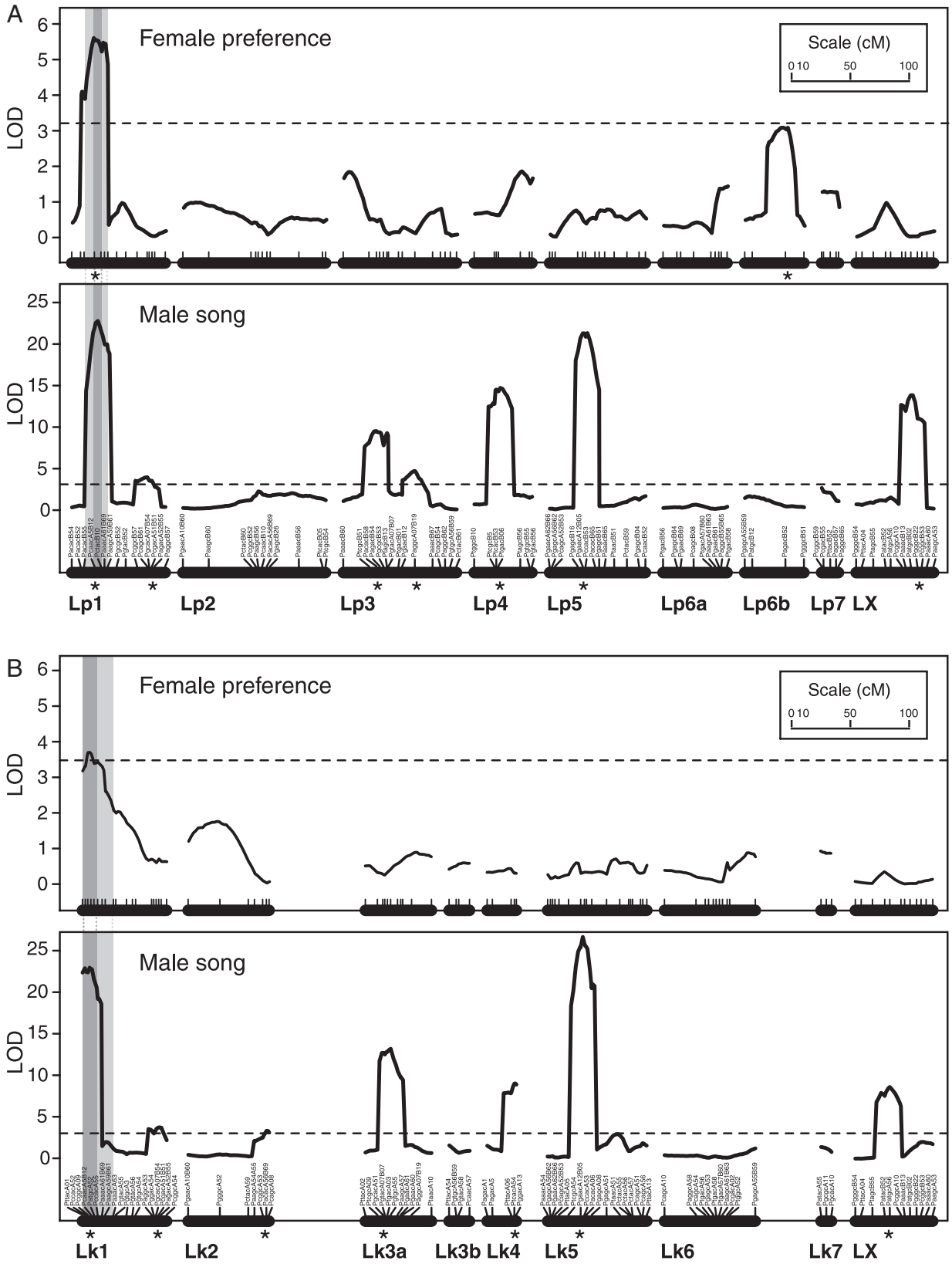

Fig. 2. QTL scans of interspecific song and preference variation on Lp (*A*) and Lk (*B*) linkage maps. Horizontal bars, with tick marks indicating AFLP marker locations, represent linkage maps. Orthology between Lk and Lp linkage groups was previously established (Fig. S1) (23). LOD traces are CIM QTL scans based on multiple imputation with a window size of 20 cM (Lp song and preference, Lk song), and IM QTL scan (Lk preference). Asterisks indicate background markers used as covariates in CIM analyses, and dashed horizontal lines indicate 5% LOD significance thresholds based on permutation. Light and dark gray vertical bars indicate the 1.5 LOD significance thresholds for location of preference and corresponding pulse rate QTL, respectively. [Reproduced with permission from ref. 23 (Copyright 2007, Wiley-Blackwell).]

Table 1. QTL results for male song and female acoustic preference

Phenotype	Linkage group	QTL location, cM	LOD	Closest marker	Marker location, cM	Phenotypic value* AA	AB	BB	QTL effect, pps ± SD	QTL effect, % parental difference	% F_2 variance explained
Preference	Lk1	6.00	3.70	PcaacA5B12	7.20	1.84	2.09	2.73	0.44 ± 0.11	14.6	47.7
Song	Lk1	6.00	13.06	PcaacA5B12	7.20	1.54	1.89	2.10	0.28 ± 0.04	9.3	18.9
Preference	Lp1	18.00	3.60	PcaacA5B12	19.24	1.83	2.10	2.74	0.45 ± 0.11	15.0	47.1
Song	Lp1	22.00	22.79	PcaacA5B12	19.24	1.55	1.88	2.10	0.27 ± 0.04	9.1	18.3

QTL results for pulse-rate preference (this study) and song (23), showing the location and peak LOD score for each QTL, closest marker to the LOD peak, marker location, mean phenotypic value for each genotype class, and QTL effect estimates (see *Results*). All QTL effects were significantly different from 0. pps, pulses per second.

**L. paranigra* and *L. kohalensis* alleles are designated A and B, respectively.

studies are similar to this one in that the signal and response traits are expressed during mate choice and likely contribute to sexual isolation between closely related species. However, both of these findings differ from ours in that males respond to a signal produced by, but not limited to, females. In our study, males produce sex-limited signals and responses by females, representing conditions for sexual selection by female choice that have long been invoked in explanations of male display trait elaboration and speciation by sexual selection (7–9).

A compelling explanation consistent with our results is that male signal and female preference are genetically coupled (1), where the same genes control variation in both behaviors. In the Lk map, the estimated location of the song and preference QTL is the same, while the Lp analysis placed them 4 cM apart. In both analyses, the 1.5 LOD confidence intervals for the preference QTL (representing a 20 cM region) contain the 1.5 LOD confidence intervals for the song QTL (representing a 10 cM region) (Fig. 2). Genetic coupling suggests a mechanism for how signals and preferences might stay coordinated as species diverge, because selective pressure keeping sexual communication behaviorally coupled would be aided by common genetic factors. When a new mutation affects the phenotype of one sex, it simultaneously affects the complementary phenotype of the other sex, facilitating behavioral coupling through mutation and pleiotropy.

An alternate explanation consistent with our results is that tightly linked, but separate, loci control song and preference variation. While our analysis colocalizes a song and a preference QTL, many genes undoubtedly reside in this region of the genome. Until the genes underlying these QTL are known, we cannot rule out the possibility of close physical linkage between genes underlying male and female behavioral traits. However, the evolutionary implications of tight physical linkage are equally profound; physical linkage can contribute to the origin of genetic correlations that promote the coevolution of male–female sexual communication in addition to that generated through assortative mating (14). At present, many more song than preference QTL have been detected (Fig. 2) (23). Our results do not explain all of the preference variation segregating between our focal species and it is likely that more preference QTL remain undetected. Ultimately, research may reveal that the genetic architecture of song and acoustic preference include both linked and unlinked loci.

Under either hypotheses of close physical linkage or pleiotropy, our data allow us to make a unique observation about the genetic process of signal-preference coevolution. Genetic correlations driving signal-preference divergence could be achieved most consistently through the substitution of comparably sized pulse-rate and pulse-rate-preference allelic variants. In the present study we found a preference QTL of moderate size (≈15% of the species difference) colocalizing with a song QTL of moderate size (≈9% of the species difference; Table 1). The experimental challenges of assaying preferences between species with such large differences in behavioral trait values likely limited our power to detect QTL of smaller size. Theory suggests that our modest sample size for preference could have yielded an overestimate, but likely not an underestimate, of the true QTL effect size (28, 29). Thus, while additional preference QTL undoubtedly exist, they are also likely to be small to moderate in size. Additional song QTL underlying the pulse rate differences between *L. paranigra* and *L. kohalensis* are each of small to moderate effect (≈5–10%) (23). Altogether, our results suggest that genetic changes in both signal and preference are capable of evolving by a mode of mutual evolution in relatively small steps. Combined with the evidence that directional selection has caused song to diverge (23), this genetic model underlying song and preference differences between species also explains the observed behavioral coupling among geographically variable populations of *L. cerasina*, a closely related species (15, 27).

Acoustic communication in crickets shows widespread (*i*) behavioral coupling, where male and female traits are matched within species; (*ii*) temperature coupling, where cooler males produce slower songs that are preferred by cooler females (5); and (*iii*) developmental coupling where males reared at cool temperatures sing slower songs that are preferred by females reared at equally cooler temperatures (30–32). Several authors have hypothesized mechanisms for both the physiological and genetic bases of behavioral coupling (3, 5, 6, 33). At the physiological level, 2 models have been contrasted (33–36). The first hypothesizes that female auditory neurons form a filter that is sensitive to the temporal pattern of conspecific male song. The alternative model hypothesizes the presence of a common neural oscillator, or central pattern generator (CPG), in males and females. In males the CPG controls rhythmic song output. In females the CPG output produces a neural template whose activity is triggered by a matching male song pattern (33). Regardless, it now seems well established that song pattern in Orthoptera is generated by a neuronal network housed in the thoracic ganglia, whereas temporal signals require processing in the brain (36), casting doubt on the hypothesis that the same neuronal substrate controls both song and preference variation. Common genes could nevertheless be expressed in different neuronal networks that generate male and female behaviors (3). Likely candidates include ion channel and neurotransmittor gene families whose members show widespread expression in the nervous system.

Behavioral coupling has been observed in a variety of sexual signaling systems and sensory modalities. Genetic correlations generated through sexual selection and/or assortative mating without the benefits of physical linkage must certainly operate in many sexual systems. Although the genetic coupling hypothesis is more easily refuted (e.g., ref. 37), our evidence for tight linkage

in an acoustic communication system, together with evidence from the visual (13) and olfactory realms (12), suggests that further genetic dissection of sexual signaling systems may prove genetic coupling to be more common. Acoustic signaling such as in birds, frogs, and many insects exemplifies the traditional sexual selection system where males signal and females respond. The evolutionary consequences of genetically mediated coordination underlying behavioral coupling has exciting and far-reaching implications for mechanisms of speciation and diversification, because divergence in sexual signaling systems creates sexual isolation as a by-product. A mutation process that affects both signal and preference could fuel rapid radiations by providing correlated genetic variation underlying both components of the mate recognition system, enabling the rapid evolution of sexual isolation.

Materials and Methods

Mapping Populations. To map QTL for acoustic preference differences between *L. paranigra* and *L. kohalensis*, we generated an interspecific F_2 hybrid population (i.e., hybrid cross 2; see ref. 23) from which a linkage map was estimated. Briefly, isofemale lines from both species were established from wild-caught females. A single *L. kohalensis* female by *L. paranigra* male pairing and subsequent mating of 23 F_1 full-sibs produced a large F_2 generation (23). Crickets were reared using published methods (20).

Phenotyping. Female phonotactic experiments were conducted in a circular arena (47-cm radius) housed in a temperature-controlled (≈20 °C) acoustic chamber, illuminated by dim red light. Single females were kept beneath a plastic cup in the center of the arena for 5 min immediately before trials, during which stimulus playback was audible. To begin a 5-min trial, the cup was lifted remotely, allowing the female to walk freely in any planar direction. In each experiment 2 songs played simultaneously, each from 1 of 2 speakers (3.5-inch in diameter, RadioShack model 40-1218) placed 180° apart on the edge of the arena. Songs were simulated digitally on a personal computer using custom software. A pulsed sinusoidal tone was generated via a 16-bit digital/analog converter (Tucker-Davis Technologies). The synthesized song was filtered at 10 kHz to prevent aliasing (Krohn-Hite filter model 3322). The songs varied in pulse rate only; pulse duration and carrier frequency does not differ significantly between *L. paranigra* and *L. kohalensis* (20). Each pulse had a 40-ms duration, 5 kHz dominant frequency, and an amplitude envelope with a rise-and-fall time of 10 and 30 ms, respectively. The speaker sound-pressure levels were monitored by using a Bruel and Kjaer SPL Meter (Type 4155) and equilibrated on a 4.0 pps pulsed tone (mean SPL across experiments: 83.3 dB) by using Tucker-Davis digital attenuators.

Laupala females and their hybrids display ''unimodal'' acoustic preference (21, 27) and respond preferentially to pulse rates within the pulse rate range (pure species) or between the range (hybrids) of the parental species. To assess individual preferences, pairs of pulse rates were offered to each female in 3 stages, designed to narrow down the response range of individual females to finer degrees with sequential stages of testing. Overall, trials spanned the range below and above the mean pulse rates for *L. paranigra* (≈0.9 pps) and *L. kohalensis* (≈3.7 pps). In the first stage, females were offered 1.7 vs. 2.7 pps. Females responding to either 1.7 or 2.7 pps in the first stage were presented with a second stage trial of either 1.2 vs. 2.2 pps or 2.2 vs. 3.2 pps, respectively.

In the third stage, females were trialed in 1 of 6 series depending on responses in Stage 2 (Table S1). Stage 3 trials differed by 0.5 pps, and were presented in a randomized order, 2 per day for 3 days. A randomization procedure determined which speaker played the faster song in a given trial. In all trials, a female approaching within 10 cm of a speaker during the 5-min trial period was considered responsive to that song. Temperature variation across all trials was minor (temperature range: 19.6–20.7 °C; mean ± SD = 20.0 ± 0.2 °C). Preferences were estimated only for the individual females that showed a typical response profile, preferring a faster pulse rate choice at the lower end of the trial range and a slower pulse rate choice at the higher end of the range. The midpoint at which a female switched from choosing the faster pulse rate to choosing the slower pulse rate determined her preference estimate (Table S1). For low-responding females, Stage 3 was repeated and results were averaged by trial. The pulse rate preference distribution was tested for deviation from normality with the Shapiro–Wilk test.

The recording and quantification of song variation was previously published (23). Briefly, a single recording per male was made during daylight hours in a temperature controlled, anechoic chamber (temperature range: 19.9–20.8 °C; mean ± SD = 20.3 ± 0.2 °C) with a Marantz PMD-430 cassette recorder and Telex microphone from screen/plastic chambers. The unfiltered songs were digitized by using Soundscope/16 software digitizing technology (GWI Instruments) at 44.1 kHz. The oscillogram plots displayed trains of pulses comprising a single male song bout, and from that, 5 pulse-period measurements were made (the beginning of one pulse to the beginning of the following pulse). The pulse-period measurements were accurate to 10 ms. Data were transformed to pulse rates (the inverse of pulse period); the F_2 hybrid song distribution was tested for deviation from normality with the Shapiro–Wilk test.

Genotyping and Linkage Mapping. Surveys for AFLPs were conducted as described in ref. 23. DNA was extracted by standard methods. Samples were diluted to 250 ng/μL for AFLP analysis and reactions followed the manufacturer protocols (GIBCO BRL AFLP starter primer kit; Life Technologies) (38). Briefly, genomic DNA was digested (PstI/EcoRI) and adaptors ligated in single reactions. Both preselective (primers with 1 additional base pair) and selective (primers with 3 additional base pairs) PCR reactions were held in a total volume of 10 μL. Adapter-ligated DNA was used as a template in preselective reactions, and preselective reaction product was used in selective reactions. We visualized AFLP fragment patterns and size standards (GS-500 ROX, Applied Biosystems) by using an ABIPrism 3100 DNA Analyzer (Applied Biosystems). AFLP markers were scored for presence/absence from the ABI-3100 trace files by using GeneScan Analysis 3.7 and Genotyper 2.5 software (Applied Biosystems).

Linkage analysis and map construction were reported in ref. 23. Briefly, AFLP markers homozygous in the parents were scored in F_2 hybrids and examined for fit to Mendelian expectations. Genotypes at several ''candidate'' codominant AFLP loci were inferred from the banding patterns (indels can result in transdominant linked AFLPs that represent the same genomic location) and assessed for fit to Mendelian expectations. Several candidate codominant alleles were isolated, sequenced, and assembled using Sequencher 4.0 to confirm homology (Gene Codes) (39).

We constructed parental linkage maps independently (Lp and Lk maps) because only estimates of linkage among recessive alleles in phase can be observed reliably with dominant markers such as AFLP. However, we included several codominant loci in each analysis to facilitate the alignment of orthologous, autosomal linkage groups between parental maps. Because male *Laupala* are X0, the genotype of X-linked dominant markers is unambiguous, allowing estimation of a single X linkage group.

Linkage analyses were performed using JoinMap 3.0 (40). The linkage groups were estimated using the JoinMap grouping module by identifying groups of markers that showed stable association across LOD thresholds. Marker order within groups was estimated by using the Kosambi mapping function of the JoinMap mapping module. Markers with identical segregation patterns were removed and the ripple function was used to test optimal marker order. Identification of orthologous linkage groups between the Lp and Lk maps (based on codominant markers included in each map) were used to construct the *Laupala* linkage map (Fig. S1) (23).

QTL Analysis. By using the R/qtl package (41), we conducted IM and CIM analyses of preference based on multiple imputation, as published for song in ref. 23. QTL scans were performed at 2 cM intervals, and the 5% experiment-wide error threshold for each analysis was determined by permutation (25).

Peaks that crossed the 10% permutation threshold in IM were designated as covariates in CIM and QTL locations and were refined as described in ref. 23. A CIM trace was produced by iteratively removing the covariate representing a given QTL within the 20 cM window surrounding the peak LOD score for that QTL.

The phenotypic effect of one allele at a given QTL was estimated in R/qtl at the marker closest to the peak LOD from regression of phenotype on genotype. From this additive allelic effect we estimated the direction and size of effect for song and preference QTL. We further calculated the phenotypic effect of each QTL as the percentage of the parental difference (3.01 pps) and the percentage of the F_2 variance explained by that QTL in an additive model ANOVA in R/qtl (41) (Table 1).

ACKNOWLEDGMENTS. We thank M. Martin and R. Yu for assistance with data collection, R. Hoy, P. Danley, J. Grace, C. Wiley, C. Ellison, S. Anderson, and members of the K.L.S. laboratory for helpful discussions and comments on this work, and 2 anonymous reviewers for insightful comments that improved the quality of this manuscript. This work was supported by National Science Foundation Grant DEB-9729325 (to K.L.S.).

1. Alexander RD (1962) Evolutionary change in cricket acoustical communication. *Evolution* 16:443–467.
2. Hoy RR, Hahn J, Paul RC (1977) Hybrid cricket auditory behavior: Evidence for genetic coupling in animal communication. *Science* 195:82–84.
3. Butlin RK, Ritchie MG (1989) Genetic coupling in mate recognition systems: What is the evidence. *Biol J Linn Soc* 37:237–246.
4. Boake CRB (1991) Coevolution of senders and receivers of sexual signals: Genetic coupling and genetic correlations. *Trends Ecol Evol* 6:225–227.
5. Gerhardt HC, Huber F (2002) *Acoustic Communication in Insects and Anurans*: *Common Problems and Diverse Solutions* (Univ of Chicago Press, Chicago).
6. Greenfield MD (2002) *Signalers and Receivers*: *Mechanisms and Evolution of Arthropod Communication* (Oxford Univ Press, London).
7. Darwin C (1871) *Sexual Selection and the Descent of Man* (Murray, London).
8. Lande R (1989) Fisherian and Wrightian theories of speciation. *Genome* 31:221–227.
9. Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. *Trends Ecol Evol* 16:364–371.
10. Ritchie MG (2007) Sexual selection and speciation. *Annu Rev Ecol Syst* 38:79–102.
11. Andersson M, Simmons LW (2006) Sexual selection and mate choice. *Trends Ecol Evol* 21:296–302.
12. Marcillac F, Grosjean Y, Ferveur JF (2005) A single mutation alters production and discrimination of *Drosophila* sex pheromones. *Proc R Soc London Ser B* 272:303–309.
13. Kronforst MR, et al. (2006) Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. *Proc Natl Acad Sci USA* 103:6575–6580.
14. Kirkpatrick M, Hall DW (2004) Sexual selection and sex linkage. *Evolution* 58:683–691.
15. Mendelson TC, Shaw KL (2002) Genetic and behavioral components of the cryptic species boundary between *Laupala cerasina* and *L. kohalensis* (Orthoptera: Gryllidae). *Genetica* 116:301–310.
16. Otte D, Alexander RD (1983) *The Australian Crickets (Orthoptera*: *Gryllidae)* (Acad of Nat Sci of Philadelphia, Philadelphia).
17. Otte D (1994) *The Crickets of Hawaii*: *Origin*, *Systematics and Evolution* (The Orthopterists' Society, Acad of Nat Sci of Philadelphia, Philadelphia).
18. Gray DA, Cade WH (1999) Quantitative genetics of sexual selection in the field cricket, *Gryllus integer. Evolution* 53:848–854.
19. Mendelson TC, Shaw KL (2005) Sexual behaviour: Rapid speciation in an arthropod. *Nature* 433:375–376.
20. Shaw KL (1996) Polygenic inheritance of a behavioral phenotype: Interspecific genetics of song in the Hawaiian cricket genus *Laupala. Evolution* 50:256–266.
21. Shaw KL (2000) Interspecific genetics of mate recognition: Inheritance of female acoustic preference in Hawaiian crickets. *Evolution* 54:1303–1312.
22. Parsons YM, Shaw KL (2001) Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. *Mol Ecol* 10:1765–1772.
23. Shaw KL, Parsons YM, Lesnick SC (2007) QTL analysis of a rapidly evolving speciation phenotype in the Hawaiian cricket *Laupala. Mol Ecol* 16:2879–2892.
24. Petrov DA, Sangster TA, Johnston JS, Hartl DL, Shaw KL (2000) Evidence for DNA loss as a determinant of genome size. *Science* 287:1060–1062.
25. Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* 138:963–971.
26. Manichaikul A, Dupuis J, Sen S, Broman KW (2006) Poor performance of bootstrap confidence intervals for the location of a quantitative trait locus. *Genetics* 174:481–489.
27. Shaw KL, Herlihy DP Acoustic preference functions and song variability in the Hawaiian cricket Laupala cerasina. *Proc R Soc London Ser B* 267:577–584, 2000.
28. Beavis WD (1998) QTL analyses: Power, precision and accuracy. *Molecular Dissection of Complex Traits*, ed Paterson AH (CRC Press, Cleveland), pp 45–162.
29. Broman KW (2001) Review of statistical methods for QTL mapping in experimental crosses. *Lab Animal* 30:44–52.
30. Olvido A, Mousseau TA (1995) The effect of rearing environment on calling song plasticity in the striped ground cricket. *Evolution* 49:1271–1277.
31. Walker TJ (2000) Pulse rates in the songs of trilling field crickets (Orthoptera: Gryllidae: Gryllus). *Ann Entomol Soc Am* 93:565–572.
32. Grace JL, Shaw KL (2004) Effects of developmental environment on signal preference coupling in a Hawaiian cricket. *Evolution* 58:1627–1633.
33. Hoy RR (1992) Genetic and temperature coupling between sender and receiver in acoustic biocommunication systems. *Semin Neurosci* 4:377–383.
34. Hennig RM (2003) Acoustic feature extraction by cross-correlation in crickets? *J Comp Physiol A* 189:589–598.
35. Nabatiyan A, Pulet JFA, de Polavieja G, Hedwig B (2003) Temporal pattern recognition based on instantaneous spike rate coding in a simple auditory system. *J Neurophysiol* 90:2484–2493.
36. Hedwig B (2006) Pulses, patterns and paths: Neurobiology of acoustic behaviour in crickets. *J Comp Physiol A* 192:677–689.
37. Lofstedt C, Hansson BS, Roelofs W, Bengtsson BO (1989) No linkage between genes controlling female pheromone production and male pheromone response in the European corn borer, Ostrinia nubilalis Hubner (Lepidoptera: Pyralidae). *Genetics* 123:553–556.
38. Vos P, et al. (1995) AFLP: A new technique for DNA-fingerprinting. *Nucleic Acids Res* 23:4407–4414.
39. Parsons YM, Shaw KL (2002) Mapping unexplored genomes: A genetic linkage map of the Hawaiian cricket Laupala. *Genetics* 162:1275–1282.
40. Van Ooijen JW (2006) LOD significance thresholds for QTL analysis in experimental populations of diploid species. *Heredity* 83:613–624.
41. Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19:889–890.