

# MULTIPLE GENETIC LINKAGES BETWEEN FEMALE PREFERENCE AND MALE SIGNAL IN RAPIDLY SPECIATING HAWAIIAN CRICKETS

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Diverging sexual communication systems can lead to the evolution of new species that no longer recognize each other as potential mates. The coevolution of male and female components of sexual communication is facilitated by physical linkage between genes underlying signals and preferences. By crossing two closely related Hawaiian crickets (*Laupala kohalensis* and *Laupala paranigra*) with vastly different song pulse rates and female preferences, and assessing segregation of songs and preferences among second generation backcrosses, we show a strong genetic correlation between song and preference variation. Furthermore, multiple, but not all, quantitative trait loci underlying song variation also predict female preferences. This physical linkage or pleiotropy may have facilitated the striking diversification of pulse rates observed among *Laupala* species in conjunction with one of the most rapid species radiations so far recorded.

**KEY WORDS:** Courtship, genetic correlation, laupala, sexual selection, speciation

By causing divergence in sexual communication systems between populations, sexual selection is thought to be a driving force in the evolution of new species (Barraclough et al. 1995; Gray and Cade 2000; Mendelson 2003). Despite some support for this idea from a range of theoretical, comparative, and empirical studies, the overall importance of sexual selection for speciation is currently unclear (Panhuis et al. 2001; Ritchie 2007). Rapid evolution of sexual communication is impaired by the functional necessity to coordinate male signals and female preferences (Butlin and Ritchie 1989). Genetic associations among behavioral components of sexual communication (e.g., male sexual signals and female preferences) facilitate divergence by transferring the effects of natural and sexual selection on some behaviors to others, allowing the coordinated evolution of the entire communication system (Butlin and Ritchie 1989; Boake 1991). Classical models of sexual selection assume that loci underlying female preferences and male traits are independent, and that associations between them (linkage disequilibrium) arise through assortative mating

(Fisher 1930; Lande 1981; Hamilton and Zuk 1982). Although linkage disequilibrium is regularly observed in nature (Bakker and Pomiankowski 1995), it is dependent on ongoing assortative mating, which can be disrupted by limited mate availability or reduced choosiness arising from poor female condition (Hunt et al. 2005; Qvarnström et al. 2006). More stable genetic associations conducive to speciation are formed either when genes underlying preference are located within the genome nearby those determining male signals (i.e., physical linkage), or when the same genes code for both traits (pleiotropy) (Verzijden et al. 2005; Bolnick and Fitzpatrick 2007). Such genetic architectures ensure that variation in both components of sexual communication is inherited together. Although the idea of genetic coupling between signal and receiver through pleiotropy has an extensive history (Alexander 1962; Butlin and Ritchie 1989), evidence for it is currently limited (but see Marcillac et al. 2005; Kronforst et al. 2006). However, this lack of empirical support does not necessarily reflect the rarity of the phenomenon, but rather that logistic difficulties associated

with assessing female preferences (Wagner 1998) have inhibited widespread testing for such associations.

The Hawaiian cricket genus *Laupala* (Orthoptera: Gryllidae) represents one of the most rapid species radiations currently known, and the fastest so far recorded for any invertebrate (Mendelson and Shaw 2005). In conjunction with this rapid speciation is a widespread diversification in the songs produced by males to attract mates (Otte 1994). *Laupala* songs consist of a simple series of pulses produced using specialized structures on the forewings. Pulse rates are highly consistent within species, but strongly divergent between species. Unimodal preferences of females for the pulse rates of conspecific males constitute a powerful barrier against hybridization between species (Shaw 2000; Mendelson and Shaw 2002). This variation is exemplified by the closely related *Laupala paranigra* (mean pulses per sec [pps]  $\pm$  SD =  $0.71 \pm 0.08$ ) and *L. kohalensis* (mean pps  $\pm$  SD =  $3.72 \pm 0.13$ ), which can hybridize under laboratory conditions to provide insights into the genetic basis of the variation distinguishing them (Shaw et al. 2007). This pulse rate variation appears to have an additive genetic basis, with several identified quantitative trait loci (QTL) of small effect, each accounting for approximately 8% of the difference between the two species (Shaw et al. 2007). Furthermore, a similar analysis of females' preferred pulse rates identified a single preference QTL that mapped to the same location as one of the song QTL (Shaw and Lesnick 2009). Although this represents one of very few known cases of tight linkage or pleiotropy between a signal and preference locus, these loci account for only a small percentage (9.1% of pulse rate and 15% of preference) of the variation between the two species (Shaw et al. 2007; Shaw and Lesnick 2009), and thus it is unclear whether this single case of linkage has a notable impact on the capacity of *Laupala* to diversify and speciate. However, the power of this previous study to detect QTL for female preference was limited by sample sizes, due to the logistic difficulty of assessing preference. As a result, it is unclear whether linkage is specific to this single pair of song and preference loci or whether it is more widespread.

Given that multiple, additive genes underlie song variation, there are two patterns predicted by widespread physical linkage or pleiotropy in a cross between *L. paranigra* and *L. kohalensis*. First, because first generation ( $B_1$ ) backcross hybrids inherit a variable number of alleles for slow pulse rate, if pleiotropy or physical linkage is prevalent, we expect a positive correlation between the songs of their sons and the preferences of their daughters. The strength of this correlation should exceed that expected from the inheritance of varying amounts of total *L. paranigra* genome in the different backcross families. Second, the presence of species-specific alleles at QTL underlying song variation should also predict female preference. In this study, we examine both of these predictions to test the hypothesis that linkage is a widespread characteristic of song and preference loci in *Laupala*.

## Materials and Methods

### CROSSING DESIGN

Species-specific, isofemale lines of *L. paranigra* (from the Kaiwika population on the southeastern slope of Mauna Kea) and *L. kohalensis* (from the Kupehau population, Kohala Mountains) were established in the laboratory and reared as described previously (Shaw 1996). A single male *L. paranigra* was crossed with a female *L. kohalensis* to generate  $F_1$  hybrids. Three  $F_1$  females (inheriting one X chromosome from each species, as females are XX) and two  $F_1$  males (inheriting an X chromosome from *L. kohalensis* only, as males are XO) were backcrossed to a single *L. kohalensis* line. Five randomly chosen female offspring from each backcross family were backcrossed again with males from the same *L. kohalensis* line as in the previous generation to produce a total of 25  $B_2$  families. Only a subset of these families was large enough to allow us to collect phenotypic data from both males and females ( $N = 12$ ), although data on one of the two sexes were gathered from additional lines, such that the sample sizes in the QTL analyses (202 males, 47 females) exceeded those in the phenotypic correlation analyses (150 males, 44 females).

The reasons for focusing on  $B_2$  individuals were manifold. First generation hybrids should be genetically identical and heterozygous at loci showing fixed differences between the two species. Quantitative phenotypes in second-generation hybrid families ( $B_1$  and  $F_2$ ) will show segregation, but the expected mean phenotypes will be the same. Third or later generation hybrid ( $B_2$  and  $F_3$ ) families are expected to vary in mean phenotype, and thus a correlation between song and preference within families might be observed if linkage or pleiotropy between these traits exists. Furthermore, examining backcross, instead of  $F_3$ , hybrids ensured that any observed *L. paranigra* markers in hybrids were in a heterozygous state (X-linked markers in males are the exception). In  $F_3$ s, *L. paranigra* markers may indicate either homozygous or heterozygous genotypes at a given QTL locus, complicating the estimation of effect sizes of alleles. Relative to  $F_3$ s, a backcross also manifests reduced phenotypic variation across all individuals, allowing for greater precision in the estimation of preference (see below).

### PHENOTYPING

A single recording of each male's song was obtained using a SONY WMD6C (Minato, Tokyo, Japan) professional walkman cassette recorder and Sony microphone. Songs were digitized using Soundscape/16 digitizing software (GWI Instruments, Somerville, MA). Pulse rate was calculated as the inverse of the mean of five measurements of the period between the start of one pulse and the start of the next (accurate to 1 ms). All recordings were carried out at  $20^\circ\text{C} \pm 0.2$  (SD).

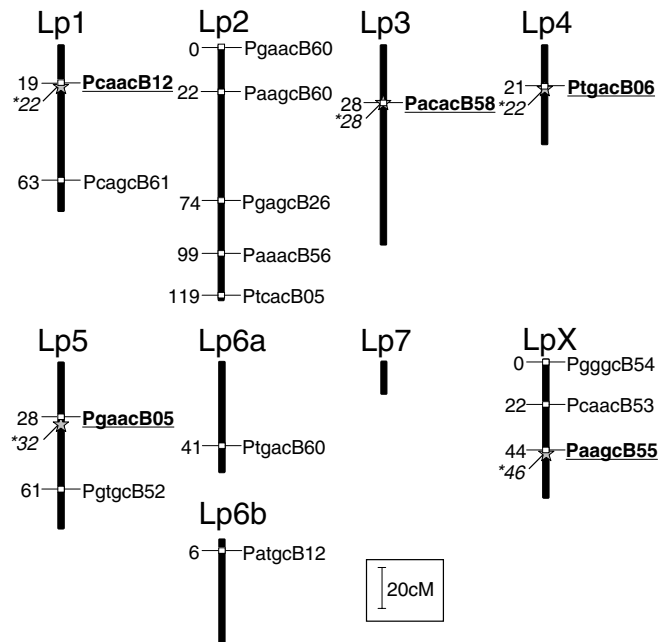
To estimate pulse rate preference, trials were conducted within a soundproof, anechoic chamber at  $20^\circ\text{C}$ . Virgin females

experienced six two-stimuli trials in *c.* 1 m diameter arena (for a more detailed description of the arena, see Shaw and Herlihy 2000). We broadcast digitally generated songs to females encompassing the range of pulse rates observed among  $B_2$  males: (1) 2.5 versus 3.0 pps, (2) 2.7 versus 3.2 pps, (3) 2.9 versus 3.4 pps, (4) 3.1 versus 3.6 pps, (5) 3.3 versus 3.8 pps, and (6) 3.5 versus 4.0 pps. The speaker that played the fast or slow song was randomized to prevent any effect of side bias. Trials were carried out from 5 h after lights-on until 10 h after lights-on, corresponding to the period when males sing (Danley et al. 2007). Trials with a given female were performed in a random order, at least 1 h apart. Trials between which females changed from approaching the faster to the slower of the two stimuli were used to infer preference (estimated as the midpoint of possible values). For example, females that preferred 3.2 pps in trial 2, but 2.9 pps in trial 3, had an estimated preference of 3.05 pps. If females always preferred the slower or faster of the two options presented, additional trials were offered in the respective direction (i.e., 2.1 vs. 2.6 pps or 3.7 vs. 4.2 pps). To exclude females that approached the speakers by chance, females with inconsistent choices (e.g., preferring 2.7 in trial 2, but 3.4 in trial 3) and those that failed to respond to at least four trials were omitted from analyses. As a basis for comparison, we used the same methodology to assess song (10 males) and preferences (six females) from the *L. kohalensis* line used for generating backcrosses. The same methodology could not be applied to *L. paranigra*, because their preferences lie well outside the variation in preference tested in this study. Females stop responding following excessive testing (C. Wiley, pers. obs.), and for the experimental trials to approach values representative of *L. paranigra* (Shaw 2000), at least 10 trials per female would be required in addition to the six listed here.

## GENOTYPING

We selected AFLP markers unique to *L. paranigra* that were closest to the peak LODs pertaining to each song QTL (Shaw et al. 2007). In this previous study, two song QTL (locations: 64cM on linkage group 1 [Lp1] and 60.9cM on linkage group 3 [Lp3]) had significant but low LOD scores (Shaw et al. 2007). Because of our reduced confidence in the location of these QTL, we considered only the remaining five song QTL in this study. We examined whether each phenotyped  $B_2$  individual possessed *L. paranigra* markers near these five male pulse rate QTL. Markers and linkage groups (i.e., chromosome) containing them are shown in Figure 1 and Table 1. DNA extraction and AFLP protocols have been described previously (Shaw et al. 2007).

A positive correlation between song and preference across backcross families need not necessarily be generated by linkage between song and preference loci. If preference and song are highly polygenic, introgression of different amounts of the *L. paranigra* genome could have a similar effect on both traits. We



**Figure 1.** Genomic map of *L. paranigra* (from Shaw et al. 2007), showing the location of AFLP markers linked to song QTL (bold, underlined), and markers outside the song QTL regions (regular font) used to assess background amount of the *L. paranigra* genome present in backcrosses to *L. kohalensis*. Stars indicate locations of song QTL as estimated from maximum LOD scores. The 95% confidence intervals surrounding these locations (estimable by  $\pm 1.5$  LOD of the peak LOD: Manichaikul et al. 2006) were cM18–25 (Lp1), cM22–40 (Lp3), cM18–28 (Lp4), cM26–34 (Lp5), and cM44–50 (LX). All eight linkage groups (corresponding to chromosomes) are shown, even though not all contained markers used in the current study. Each linkage group is shown to scale, and the marker locations (to the nearest cM) are given on the left side of each linkage group.

examined this alternative explanation by screening all females at a number of markers outside the QTL regions. We selected markers that were at least 20 cM away from the peak LOD scores for each QTL and at least 20 cM away from one another, to ensure that each marker was inherited more or less independently. Because the markers in the original song QTL study by Shaw et al. (2007) were generated from a single individual of either species, and because the two species are closely related, a large proportion of the markers that were used for making the original linkage maps are not species specific (i.e., are polymorphic in one or both species). Because the current study involved different parental individuals, only a subset of the original markers proved informative. In total, we identified 11 markers, situated across five of the eight linkage groups (see Fig. 1 for the identity and location of markers used), that conformed to our spacing requirements. If overall amount of *L. paranigra* genome is indeed underlying any correlation between the two traits, mean female preference across families is

**Table 1.** Effects of *L. paranigra* alleles at QTL underlying song differences on the songs and preferences of B<sub>2</sub> backcrosses to *L. kohalensis*. Effect sizes were determined by separate ANOVA for the two sexes, and reflect the decrease in pulse rate caused by one *L. paranigra* allele in a heterozygous state. Significant effects are indicated in bold. The rightmost column tests the null hypothesis that QTL had a similar effect on male song and female preference. Sample sizes refer to the number of males and females that possessed each of the QTL, although all 202 males and 47 females are included in the analyses. When each of the 11 markers unlinked to song QTL is included in an ANOVA with the five QTL markers above, none predict female preference (all but one marker had  $P > 0.1$ ). One marker (PaacB56) had a  $P$ -value of 0.094.

| Linkage group | Marker near QTL* | Previous QTL effect on song* | QTL effects in current study |                             |                       |            |                             |                       | Interaction sex × QTL on pulse rate |
|---------------|------------------|------------------------------|------------------------------|-----------------------------|-----------------------|------------|-----------------------------|-----------------------|-------------------------------------|
|               |                  |                              | song                         |                             |                       | preference |                             |                       |                                     |
|               |                  |                              | <i>N</i>                     | pps (95% CI)                | % parental difference | <i>N</i>   | pps (95% CI)                | % parental difference |                                     |
| Lp1           | PaacB12          | 0.274                        | 22                           | <b>0.292 (0.198, 0.386)</b> | 9.4                   | 6          | <b>0.530 (0.279, 0.783)</b> | 17.6                  | $P=0.067$                           |
| Lp3           | PacacB58         | 0.149                        | 65                           | <b>0.203 (0.145, 0.261)</b> | 6.7                   | 13         | 0.114 (−0.083, 0.310)       | 3.8                   | $P=0.636$                           |
| Lp4           | PtgacB06         | 0.206                        | 19                           | <b>0.173 (0.067, 0.278)</b> | 5.7                   | 2          | −0.413 (−0.877, 0.052)      | n/a                   | <b><math>P=0.001</math></b>         |
| Lp5           | PgaacB05         | 0.289                        | 63                           | <b>0.351 (0.289, 0.414)</b> | 11.7                  | 12         | <b>0.243 (0.045, 0.440)</b> | 8.1                   | $P=0.285$                           |
| LX            | PaagcB55         | 0.239                        | 42                           | 0.047 (−0.025, 0.118)       | 1.6                   | 7          | 0.110 (−0.119, 0.340)       | 3.7                   | $P=0.501$                           |

\*Data from Shaw et al. 2007.

expected to decline with number of *L. paranigra* alleles present, regardless of whether these alleles are within or outside song QTL regions.

## DATA ANALYSIS

To assess the relationship between the songs and preferences of B<sub>2</sub> siblings, we weighted the correlation and regression by the number of females in each family. Because the precision of our estimates was lower for females, and sample sizes were about a third of that of males, number of females was the foremost variable determining our confidence of the location of each family within two-dimensional phenotypic space. Because there is error associated with measuring both variables, and neither trait could be described as the “predictor” or “response” variable (Smith 2009), we employed reduced major axis regression rather than traditional ordinary least squares regression to estimate the slope of the relationship. We did this using the *eivreg* function in Stata version 10.

The effects of previously identified song QTL on the pulse rates of males and the preferences of females were tested by five-way analysis of variance (ANOVA) on the two sexes separately. To assess whether QTL had different effects on song and preference, we carried out an additional ANOVA whereby we tested for interactions between sex and each of the QTL on a common response variable, pulse rate.

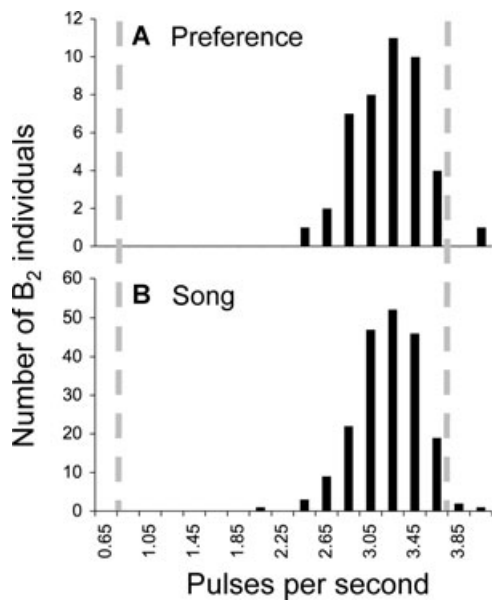
## Results

In total, the phonotactic behavior of 104 B<sub>2</sub> females were tested, of which 46 met our criteria for estimating preference. Recording

the songs of males is logistically much faster than estimating the preferences of females (carried out over two days per female), and thus the overall number of females that could be phenotyped was limited by time and their life span (only a subset could be tested in the first place). Nevertheless, 10 *L. kohalensis* females were tested using identical methodology, and the six that responded showed a mean preference that closely matched conspecific pulse rates (see Fig. 3), as well as estimates from previous studies (Shaw 2000; Mendelson and Shaw 2002). This indicates that although our sample sizes are low, the methods generate reliable estimates of mean preference for each family.

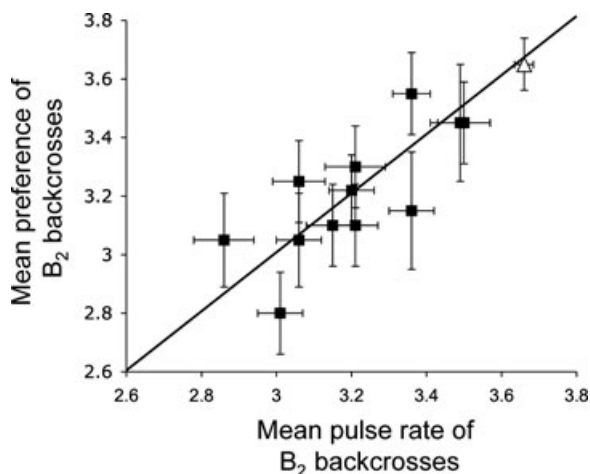
We found that the distributions of preferences and songs among B<sub>2</sub> backcrosses were similar (Fig. 2), and conformed to expectations based on an additive, quantitative genetic basis of the variation (i.e., mean phenotypes lay approximately 87.5% between the two parental species, in the direction of *L. kohalensis*). The mean song and pulse rate preference for each backcross family were all slower than the *L. kohalensis* line used in the backcrosses, implying that all families possess some *L. paranigra* alleles underlying song and preference (Fig. 3). As predicted by genetic linkage between the two traits, we detected a significant positive correlation between the pulse rates and preferences within B<sub>2</sub> families ( $R = 0.76$ ,  $P = 0.004$ ). Indeed, the slope of the reduced major axis regression ( $\beta \pm SE = 1.008 \pm 0.244$ ) was remarkably similar to that expected (i.e.,  $\beta = 1$ ) if all song loci are linked with preference loci of equivalent effect sizes (Fig. 3).

We tested whether this positive correlation can be attributed to differing amounts of background *L. paranigra* genome in the different families by determining whether there was a relationship between preference and the number of *L. paranigra* alleles

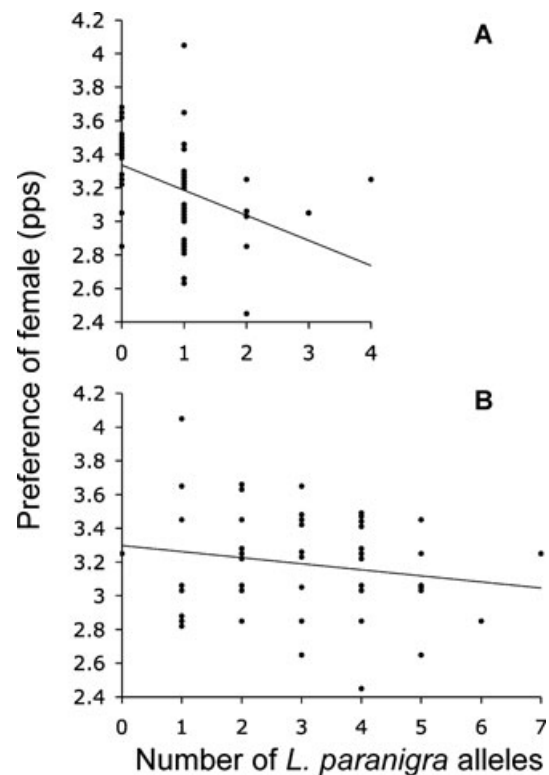


**Figure 2.** Frequency of female preferences (A) and male songs (B) among  $B_2$  backcrosses. The dashed lines indicate mean phenotypes within the two parental species, with *L. kohalensis* on the right (parental data from Shaw et al. 2007).

present. Each *L. paranigra* allele has a probability of 0.25 of being present in any one  $B_2$  individual. Consistent with this expectation, females possessed a mean of 1.02 of five possible alleles linked with QTL (expected = 1.25), and 3.04 of 11 possible alleles outside QTL regions (expected = 2.75). We found that although females preferred slower pulse rates when they possessed more



**Figure 3.** Association between the mean songs and preferences within each  $B_2$  family. Each family constitutes the offspring from a single  $B_1 \times L. kohalensis$  cross. Phenotypes are expressed in pulses per second. Error bars are standard errors based on ANOVA between  $B_2$  families. The solid line depicts a reduced major axis regression fitted to the data. The open triangle shows the mean song and preference for the *L. kohalensis* line used to generate the backcrosses, and is not included in the analysis.



**Figure 4.** Relationships between the number of *L. paranigra* alleles possessed by a  $B_2$  female and her preferred pulse rate. Panel A contains the alleles linked to the five song QTL, whereas panel B contains alleles located at least 20 cM away from song QTL (a total of 11 possible alleles). For illustrative purposes, datapoints that overlapped others in value are staggered.  $\beta$  values  $\pm$  standard errors for the two regressions (the average effect on preference of one additional *L. paranigra* allele) were  $-0.136 \pm 0.050$  (A:  $t_1 = -2.66$ ,  $P = 0.011$ ) and  $-0.034 \pm 0.029$  (B:  $t_1 = -1.13$ ,  $P = 0.266$ ). Based on the standard errors around these  $\beta$  estimates, the difference in slope was statistically significant ( $t_{89} = -1.73$ ,  $P = 0.044$ ).

*L. paranigra* markers associated with QTL, preferred pulse rates declined only slightly, and nonsignificantly, with the number of background *L. paranigra* alleles (Fig. 4).

In an effort to ensure each marker represented an independent sample of the genome, we selected markers located at least 20 cM apart, and subsequently checked for independent inheritance of loci within  $B_2$  females. Of 120 pairs of markers, 16 showed significant ( $P < 0.05$ ) correlations (i.e., coinheritance of *L. paranigra* alleles). Note that six of 120 are expected by chance. Furthermore, four of the significant correlations were negative (therefore unlikely to be real), and none of the 16 significant correlations were between markers located on the same chromosome/linkage group, suggesting that most of these significant results are Type I errors caused by the large number of comparisons with small sample sizes in each, rather than genuine examples of nonindependence. Taken together, these results suggest that nonindependent inheritance of markers is a relatively minor concern in the current study.

Next, we directly tested the effect of each song QTL on the preference of females using ANOVA. The two QTL with the largest effects on male pulse rate also significantly affected the preferences of females in the same direction (Table 1). Although the effect size of a third song QTL on preference was not significantly different from zero, it too was in the same direction and of comparable size to that observed for song. Interestingly, the QTL that was not replicated in the previous study (on the X-chromosome) failed to predict pulse rates of males (Table 1), and there was a corresponding lack of effect on preference. For only one of the five song QTL (on linkage group Lp4) were the data inconsistent with the null hypothesis that each song QTL has an equivalent effect on female preference (Table 1). However, by chance, only two females possessed the *L. paranigra* marker linked with this QTL.

To examine whether the pattern observed in Figure 3 was caused solely by the preference loci linked to the song QTL on linkage groups Lp1 and Lp5, we repeated the reduced major axis regression including only individuals lacking the *L. paranigra* markers linked to either or both of these two song QTL. Despite the smaller sample afforded by excluding these individuals, and one less family being included in the analysis (all females from this family possessed one or both markers), the correlation between the songs of sons and preferences of daughters remained significantly positive ( $R = 0.70$ ,  $P = 0.017$ ). The slope of the reduced major axis regression ( $\pm$ SE) was  $0.697 \pm 0.237$ .

## Discussion

Given the proposed prominent role of diverging sexual communication systems in the evolution of new species (Barraclough et al. 1995; Gray and Cade 2000; Mendelson 2003), there has been substantial theoretical investigation into the evolutionary and genetic mechanisms promoting such divergence. Several models have highlighted the importance of the inheritance mechanism underlying female preferences for promoting speciation when diverging populations continue to remain in contact (Ortiz-Barrientos et al. 2002; Kirkpatrick and Hall 2004; Verzijden et al. 2005; Servedio et al. 2009). These models suggest that mechanisms most conducive to speciation are those that prevent recombination between loci underlying female preferences and male signals. A tight physical linkage between preference and signal loci ensures their coinheritance, avoiding the recombination that disrupts coordinated divergence in the face of persistent hybridization. Not only does such linkage prevent recombination in the face of hybridization between populations, but also facilitates robust genetic correlations between signal and receiver within populations, and thereby increases the potential for rapid divergence through sexual selection.

A previous study identified a preference locus on linkage group 1 that is tightly linked with a song QTL underlying variation between *Laupala* species, but both these QTL accounted for a minor proportion of overall variation in song and preference (Shaw and Lesnick 2009). Our results suggest that this genetic linkage between song and preference loci is much more extensive than previously thought. In addition to corroborating the preference QTL on linkage group 1 (Lp1), our results demonstrate the presence of a second preference QTL associated with the song QTL on linkage group 5 (Lp5). Furthermore, although the precise number of linked loci in *Laupala* cannot be determined from our data, a positive genetic correlation between song and preference was observed even among individuals lacking song and preference QTL on Lp1 and Lp5, suggesting that at least three, and probably more, pairs of linked loci are involved. Thus, our findings provide evidence that several loci underlying variation in pulse rate between *L. kohalensis* and *L. paranigra* are physically linked to, or have pleiotropic effects on, preference.

Our estimated effect sizes of each of the song QTL on male pulse rate were broadly consistent with the original estimates by Shaw et al. (2007); all but one of these original estimates fell within the 95% confidence intervals of our estimates. The single exception was the QTL situated on the X chromosome (linked to marker PaagcB55), which failed to predict either song or preference. In the previous study, Shaw et al. (2007) reported highly significant statistical support for the presence of this same QTL in just one of two replicate crosses (the other four song QTL were replicated). One interpretation of these findings is that the locus on the X is polymorphic, conferring variable allelic effects, within *L. paranigra*. If so, the *L. paranigra* male used in the original cross to generate the lines presented here carried an allele with a negligible effect on the pulse rate difference between species. In addition to the QTL's effects on song, the effect size of the preference QTL located on Lp1 was also consistent with a previous estimate (0.45 pps: Shaw and Lesnick 2009). For all but one QTL (linked with marker PtgacB06 on linkage group Lp4), the effect on preference was not significantly different from the effect on song. However, the power of our study to test for such interactions is limited by our sample sizes, as evidenced by the broad 95% confidence intervals associated with the estimates of effect sizes on each trait. Consequently, although our results support the possibility that most QTL have equivalent effects on both song and preference, it is premature to conclude whether this is indeed the case.

There are two leading explanations for the extensive linkage observed in the current study. First, if the genetic correlation is caused by physical linkage between separate signal and preference loci, this implies that either sexual selection tends to favor loci that are linked, or linkage occurs often simply by chance. This latter scenario could be a feature of highly polygenic signals and preferences, because, by chance, a new preference locus

might be expected to reside near an existing song locus. If this is the case, linkage may be a widespread characteristic of highly quantitative traits. However, the lack of an association between preference and markers outside song QTL suggests that this scenario is unlikely. Instead, physical linkage may have been built up by sexual selection in *Laupala* as new loci underlying male pulse rate (or female preference) were favored when situated nearby existing loci controlling the complimentary component of sexual communication.

An alternative explanation for the genetic correlation observed is that male pulse rates and female preferences are under common genetic control. Recent data from multiple taxa have suggested that functionally similar genes often cluster within the genome (Hurst et al. 2004; Xu et al. 2008), and this has led to the suggestion that the basis for clustering might be pleiotropy (Protas et al. 2008). In a stark contrast, pleiotropy is rarely invoked to account for genetic correlations between traits under sexual selection (Bakker and Pomiankowski 1995), presumably because it is often unclear how single loci could influence such disparate traits as innate preferences and elaborate ornaments or sexual displays. However, there is reason to suspect that acoustic communication in crickets may involve a pleiotropic coupling between signal and preference (Alexander 1962; Hoy 1974; Pires and Hoy 1992). Most rhythmic behaviors of animals, including cricket song and song recognition (Hedwig 2000; Bush and Schul 2006), are under the regulation of central pattern generators (CPGs), circuits of neurons that act as pacemakers. If the same CPG underlies both male song and female preference, any genes influencing the output of this pattern generator (e.g., those regulating ion channels or synapses) would have pleiotropic effects on both traits. Even if separate CPGs control song and preference, some or all genes may have pleiotropic effects on both. Our data are consistent with the hypothesis that the CPGs underlying male pulse rate and female preference for pulse rate have a largely common genetic basis, although we cannot dismiss close physical linkage of separate genes underlying the male and female components of communication. Interestingly, other attributes of cricket song that reflect the elements being repeated (e.g., trill length in *Gryllus* and syllable number in *Ephippiger*), rather than the rate of repetition, display weak or no evidence for linkage of loci underlying male and female components of communication (Gray and Cade 1999; Ritchie 2000). This raises the possibility that in crickets only song components controlled by the cyclic pace of a CPG may be genetically linked to female preferences. Three other recent studies—of pheromonal communication in *Drosophila melanogaster* (Marcillac et al. 2005) and visual signalling in *Heliconius* butterflies (Kronforst et al. 2006) and *Oryzias* fish (Fukamachi et al. 2009)—are consistent with a hypothesis of pleiotropic genetic contributions to signal and receiver variation. All three cases suggest single pleiotropic genes

of large effect, rather than quantitative sexual signals and preferences more representative of sexual traits in general. Furthermore, all are unusual in that they represent phenotypes in which males are the sex responding to female signals. Our data from *Laupala* suggest that not only may such pleiotropy extend to additional sensory modalities (i.e., acoustic signalling), it may also apply to more conventional sexual signalling systems, involving quantitative male signals and female preferences.

Regardless of whether the observed genetic correlations between male song and female preference stem from pleiotropy or physical linkage, both genetic architectures facilitate speciation by preventing recombination between loci underlying sexual communication. Furthermore, by generating durable genetic correlations between male and female traits, either genetic architecture provides favorable conditions for Fisherian runaway processes to operate alongside other mechanisms of sexual selection (Lande 1981; Mead and Arnold 2004). Not only is the genetic correlation observed in this study made durable by the underlying genetic architecture, but it was remarkably strong. The 1:1 relationship between song and preference observed here ensures that evolution of one trait would directly cause parallel changes in the other. The extensive pleiotropy or linkage between pulse rate and preference loci in *Laupala* may thus have profound implications for the rapid diversification of sexual communication between populations, as well as serving as a robust barrier to hybridization between closely related species that arise from this process. As such, genetic architecture of sexual communication may be central to the rapid speciation and extensive sympatry that characterizes the genus (Shaw 2002; Mendelson and Shaw 2005).

A recent review has suggested that one of the primary advances in our understanding of sexual selection since Darwin has come from our enhanced understanding of the evolution of the largely female component of sexual communication, mate choice (Jones and Ratterman 2009). However, even today, logistic difficulties associated with the characterization of mate preferences (Wagner 1998) have been a chief impediment to furthering our understanding of sexual selection. For this reason, although the widespread linkage between multiple loci underlying sexual signals and preferences observed in *Laupala* is remarkable, there are too few data from other systems to determine whether it is unusual. Similar studies in other systems, especially those with contrasting rates of speciation, will reveal whether linkage is more widespread than currently appreciated, and whether the genetic architecture of sexual communication is indeed a powerful determinant of the propensity of certain evolutionary lineages to undergo speciation.

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