

Divergence of Mate Recognition Behavior and Its Consequences for Genetic Architectures of Speciation

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ABSTRACT: The divergence of premating behavior and morphology plays a primary role in speciation, and an understanding of the genetic architectures of these phenotypes is essential for the evaluation of models of the speciation process. However, our empirical knowledge of the genetics underlying speciation-related traits remains limited. In this article, we argue that a dissection of specific aspects of the genetic architecture of such traits in a comparative context can allow us to rule out some mechanisms of divergence. We discuss these ideas with reference to our investigation of intersexual communication behaviors involved in mate recognition in the Hawaiian cricket genus *Laupala*. Different species of *Laupala* sing distinctively and show species-specific acoustic preferences. We focus on the sister species *Laupala paranigra* and *Laupala kohalensis*, characterized by differences in these classic courtship phenotypes. We discuss our preliminary results on the directionality of effect of substituted alleles underlying these species differences. We then discuss these results in the context of historical inference, a necessary perspective for testing the genomic predictions made by theories of speciation that focus on evolution of mate recognition systems.

Keywords: courtship song, sexual selection, mate recognition, crickets, *Laupala*, quantitative trait loci (QTL) analysis.

Our understanding of speciation has largely come from two domains of study: first, the genetic and phenotypic study of population or species differences and, second, the investigation of evolutionary forces that cause early lineage divergence. The genetic architecture of traits in-

involved in speciation (see fig. 1) is a component of the first domain of study. Although this subject remains relatively unexplored, it has the potential to offer unique insights into processes underlying divergence. The second domain of study has given rise to numerous theories of speciation, some of which make reasonably simple genomic predictions that are becoming accessible to empirical investigation.

The limited understanding of the genetic architectures of speciation is partly due to the complexity of the problem as well as to the inaccessibility of most genomes to molecular study. For example, the importance of Type I genetic architecture (where many genes of small, additive effect underlie phenotypic variation between species; Templeton 1981) versus Type II genetic architecture (with major/modifier gene effects underlying phenotypic variation) has been debated but remains unresolved, mainly due to a lack of empirical evidence (Barton and Charlesworth 1984; Carson and Templeton 1984). Type I and II architectures depend on the magnitude and directionality of allelic effects as well as on the interaction among loci and alleles and thus represent complex categories. With advances in genome mapping and quantitative trait loci (QTL) analysis, the opportunity exists to examine some of the basic aspects of genetic architecture directly. A dissection of genetic architecture can lead to some simple measures, which puts us in the position to rule out some models of speciation in favor of others. In this article, we focus on the models of mate recognition evolution to illustrate this approach.

Darwin (1879) observed that many recently diverged taxa differ most conspicuously in secondary sexual characteristics, which suggests the early action of specific evolutionary forces on phenotypes involved in mate recognition. It is not difficult to connect the evolution of mate recognition traits to the origin of new species. Divergence in such characteristics could lead to a decrease in gene flow between daughter lineages, which would generate exclusive interbreeding or exclusive genealogical relationship

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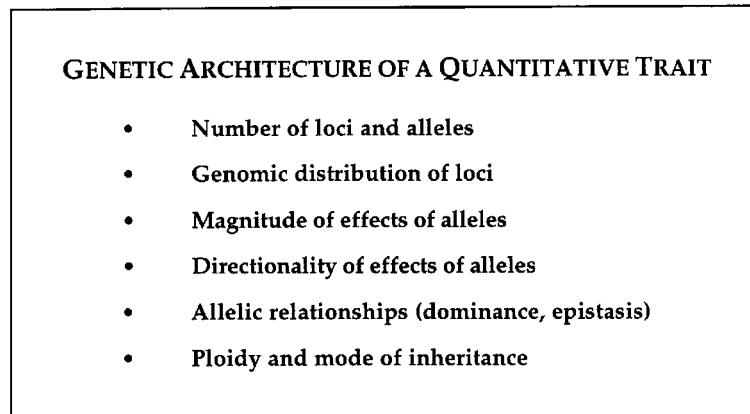


Figure 1: Different aspects that can contribute to the genetic architecture of a quantitative trait

(Shaw 1998) within two daughter populations. Accordingly, the evolution of sexual traits takes on a central role in several speciation models, either by the action of sexual selection (e.g., Fisher's [1930] runaway process) or through a combination of sexual selection and stochastic change (e.g., Kaneshiro 1976; Templeton 1980; Lande 1981; Carson 1982, 1986). The genetic mechanisms and potential outcomes of models of speciation invoking selection versus a combination of selection and drift can be quite different, however, despite their common regard for evolution in mate signaling systems.

In this article, we examine one aspect of genetic architecture that is likely to vary under different forces that can affect mate recognition phenotypes. We consider only one facet, the directionality of effect of alleles underlying species differences that were substituted during the course of speciation, in an attempt to break down the complex study of genetic architecture into a tractable question. The focus of our research, sister species of the endemic Hawaiian cricket *Laupala*, *Laupala paranigra* and *Laupala kohalensis*, are characterized by a classic courtship phenotype, the acoustical communication system of crickets (family Gryllidae). We concentrate on speciation models that involve coordinated evolution in male and female signaling systems, namely cricket song and acoustic preference behavior, alone or in concert with previous application to the geochronological setting of the Hawaiian Islands. We first discuss the directionality of effect of substituted alleles during the course of speciation predicted by these models. We then discuss our preliminary findings and the implied historical framework for testing genomic predictions.

Mate Recognition Evolution and Speciation

Fisher Process

The process of divergence by sexual selection outlined by Fisher (1930) occurs in a purely selective environment. Sexual selection by differential male mating success due to biased female choice can cause male signal evolution by establishing a genetic correlation between male and female components of the intersexual communication system. Once such genetic correlations become established, Fisher suggested that continued divergence in mate recognition signals would occur through indirect selection on female preferences correlated with extreme signals. This happens because females choosing males with extreme signals indirectly choose genes underlying female choice for extreme signals that males carry due to the genetic correlation. Eventually, the evolutionary response of the male trait to sexual selection is halted by natural selection. However, if divergence prior to this point promotes exclusive mating in the two daughter populations, the process would result in the evolution of new species. This process could occur conceivably under a Type I or II genetic architecture. However, if the traits under selection are quantitative, the process is best facilitated by Type I because it allows evolution to occur in relatively small increments, thereby enabling coordinated evolution in an intersexual signaling system (Barton and Charlesworth 1984; Butlin 1995).

Lande's Fisher Process

Lande's (1981) model of the Fisher process treats female preference as selectively neutral. Changes in female pref-

erence lead to evolution by sexual selection on male traits and further correlated change on preference itself. So, while female traits may change at first by drift and subsequently by indirect genetic correlation with male traits, male traits continue to evolve via sexual selection. This process is also aided by many genes of small, additive effect. While it is not a requirement of either Fisher's or Lande's models, the coordinated evolution of male and female traits would be facilitated by close physical linkage of these traits (popularized by Alexander [1962] in studies of the singing Orthoptera; Doherty and Hoy 1985).

Founder Models of Speciation

In founder models (e.g., Kaneshiro 1976; Templeton 1980; Carson 1982), drift can affect male or female (or both) components of the coordinated courtship system. During a brief period of low population size, drift is invoked as a dominant force due to either a relaxed sexual selective environment (specifically affecting male traits in the Kaneshiro process; Kaneshiro 1976), a disorganization/reorganization process affecting a complex genetic system of communication between males and females (Carson 1982, 1986), or a readjustment of gene frequencies at major loci affecting a coadapted complex (releasing additive genetic variance; Templeton 1980). Specific genetic architectures in founder models have been proposed only by Templeton (1980, 1996), who has argued that a Type II genetic architecture is required under the genetic transience model.

Directionality of Effects

Our expectations for the directionality of effect of substituted alleles during the course of speciation differ among the mechanisms discussed above. As pointed out by several authors (Coyne 1996; Laurie et al. 1997; True et al. 1997; and formally Orr 1998), directional selection will lead to a biased substitution of alleles into a population that causes directional change in the quantitative phenotype under selection. To see how, imagine two sister species with males that differ in antler length: in the "high" species, males have long antlers, and in the "low" species, males have short antlers. Likewise, females of the high species prefer males with long antlers, and females of the low species prefer males with short antlers. Suppose also that the most recent common ancestor to these two species had antlers and had a preference for antlers of intermediate length. Under a pure Fisher process of sexual selection, we expect that the alleles that increased the antler length in the high

lineage (i.e., plus factors) would have been favored over alleles that did the opposite. Likewise, we expect alleles that decreased the antler length in the low lineage (i.e., minus factors) to have been favored over alleles that increased antler length. This follows because sexual selection by a Fisherian mechanism will favor alleles that decrease the trait in the low line and those that increase the trait in the high line. Because female preference is evolving as a correlated response to selection on male traits, we expect the allelic effects underlying female preference to show the same bias in directionality.

In contrast, if antler length or preference differences between the two species had diverged by a random process, alleles increasing antler length or preference would not have been favored over those decreasing these phenotypes during the course of evolution of either nascent species. Thus, compared to a strict Fisher process, models invoking drift make different predictions about the proportion of plus and minus allelic effects that underlie male and female phenotypic differences. Although the high (or low) line must contain some plus (or minus) factors, evolution by drift implies no favored directionality of allelic effects on phenotype and thus predicts a mixture of alleles with both plus and minus effects in species of either extreme phenotype. Finding a high concentration of plus factors in the high line and minus factors in the low line would lead us to reject a random divergence process and to infer a role for directional sexual selection (True et al. 1997; Orr 1998). Table 1 summarizes the predictions of the directionality of allelic effect under the Fisher, Lande, and founder models.

Hawaiian Cricket Genus *Laupala*

The cricket genus *Laupala*, with 37 flightless species of crickets (Otte 1994; Shaw 2000a), is well suited to the study of speciation by the evolution of mate recognition systems. The genus is entirely endemic to the Hawaiian archipelago, a setting that provides a recent window of time through which to view the speciation process. The present diversity must therefore have arisen within the last 5 million years, the age of the oldest extant island of Kauai. The focal taxa in this study, *Laupala paranigra* and *Laupala kohalensis*, are closely related and are completely allopatric (fig. 2), with *L. kohalensis* limited to forests of the Big Island's oldest volcano, Kohala (0.43 million years old) and *L. paranigra* to the forests of the younger volcanoes of Kilauea, Mauna Loa, and Mauna Kea (0–0.4 million years old; Clague and Dalrymple 1987). Both mitochondrial and nuclear data display 0.3%–0.4% sequence divergence between these two species (Shaw 1996b; K. L. Shaw, un-

Table 1: Directionality of effect on the male and female component of the mate recognition system by alleles from the high line that were substituted during the course of evolution under different speciation mechanisms

Model	Source	Allelic directionality of effect	
		Female	Male
Fisher process	Fisher 1930	Uniform (+ or -)	Uniform (+ or -)
Lande Fisher process	Lande 1981	Mixed (+ and -)	Uniform (+ or -)
Kaneshiro process	Kaneshiro 1976	Uniform (+ or -)	Mixed (+ and -)
Disorganization-reorganization	Carson 1982	Mixed (+ and -)	Mixed (+ and -)
Genetic transience	Templeton 1980	Mixed (+ and -)	Mixed (+ and -)

Note: A plus sign indicates that substituted alleles from the high line increase the trait value; a minus sign indicates that the substituted alleles decrease the trait value. Uniform distributions (+ or -) are predicted by directional selection processes of evolution. Mixed distributions (+ and -) are predicted by random processes of change.

published manuscript). This demonstration of close relationship facilitates study of the genetics of speciation as opposed to the secondary effects that inevitably accrue through continued divergence subsequent to speciation. This is critical as we seek to avoid systems where subsequent evolution has masked the initial genetic changes accompanying speciation.

Like many other cricket species, male crickets in the genus *Laupala* use song extensively in courtship, to which sexually receptive females respond. The most closely related species of *Laupala* have distinct songs and acoustic preferences (Otte 1994; Shaw 1999, 2000b; Shaw and Herlihy 2000; Parsons and Shaw 2001), which suggests that functionally relevant phenotypes of a coordinated mate communication system vary at the earliest stages of divergence. In addition, previous studies have shown that this behavioral variation has a large genetic component (Shaw 1996a, 2000b). Acoustically distinct species of *Laupala*, including the focal taxa of this study, can be crossed in the laboratory, a requirement for interspecific studies of genetic architecture.

In addition, *Laupala* exhibit repeated patterns of acoustic behavioral evolution across the Hawaiian archipelago, an unparalleled geochronological framework. Both mitochondrial and nuclear sequence data (Shaw 1996b; K. L. Shaw, unpublished data) suggest that the current radiation began on the oldest island, Kauai (5 million years old), and radiated into younger island habitats as those islands appeared. Similar songs and song communities have been established in chronological series across the archipelago. Thus, historical replication of acoustic evolution coupled with speciation exists in this group. The youngest lineages of *Laupala* (as exemplified by *L. paranigra* and *L. kohalensis*) are but one example within this genus of an extremely closely related, interfertile species pair differing in male and female traits of the courtship system.

Genetic Architecture of Reproductive Traits in Laupala

We currently lack a detailed understanding of the genetic architecture of sexual isolation in general (Ritchie and Phillips 1998) and of mate recognition behaviors in *Laupala* in particular. We summarize our current knowledge of the genetics of acoustic communication differences between *L. paranigra* and *L. kohalensis* as learned through classical quantitative genetic studies below. We then proceed to discuss our preliminary data on genetic architecture of song derived from genome mapping and QTL analyses.

Genetic studies of cricket song conducted between *L. kohalensis* and *L. paranigra* (Shaw 1996a; fig. 2) have shown that pulse rate production is stereotyped (no learning is involved), pulse rate difference has a large genetic component (Shaw 1996a), studies in other crickets of temporal song variation show moderate to high heritability (Webb and Roff 1992; Gray and Cade 2000), and pulse rate difference is quantitative. These results concur with other classical genetic studies, which suggests that many acoustic signaling traits evolve with polygenic genetic architectures (reviewed in Ritchie and Phillips 1998).

Genetic studies of females in this species pair (Shaw 2000b) reveal that, like other systems, females are more attracted to temporal pulse structures of the song characterizing their own species. Unimodal preference functions also characterize F_1 and backcross generations, with hybrid females expressing preferences for pulse rates intermediate of parentals. In addition, pulse rate preferences segregate in the backcross generation. Mean pulse rate preference matches mean pulse rate in both parental and hybrid generations. Thus, based on F_1 hybrids and segregation patterns in backcross females, changes in both signal and receiver components of the mate recognition system are consistent with a multilocus model of change through incremental steps. The results therefore suggest

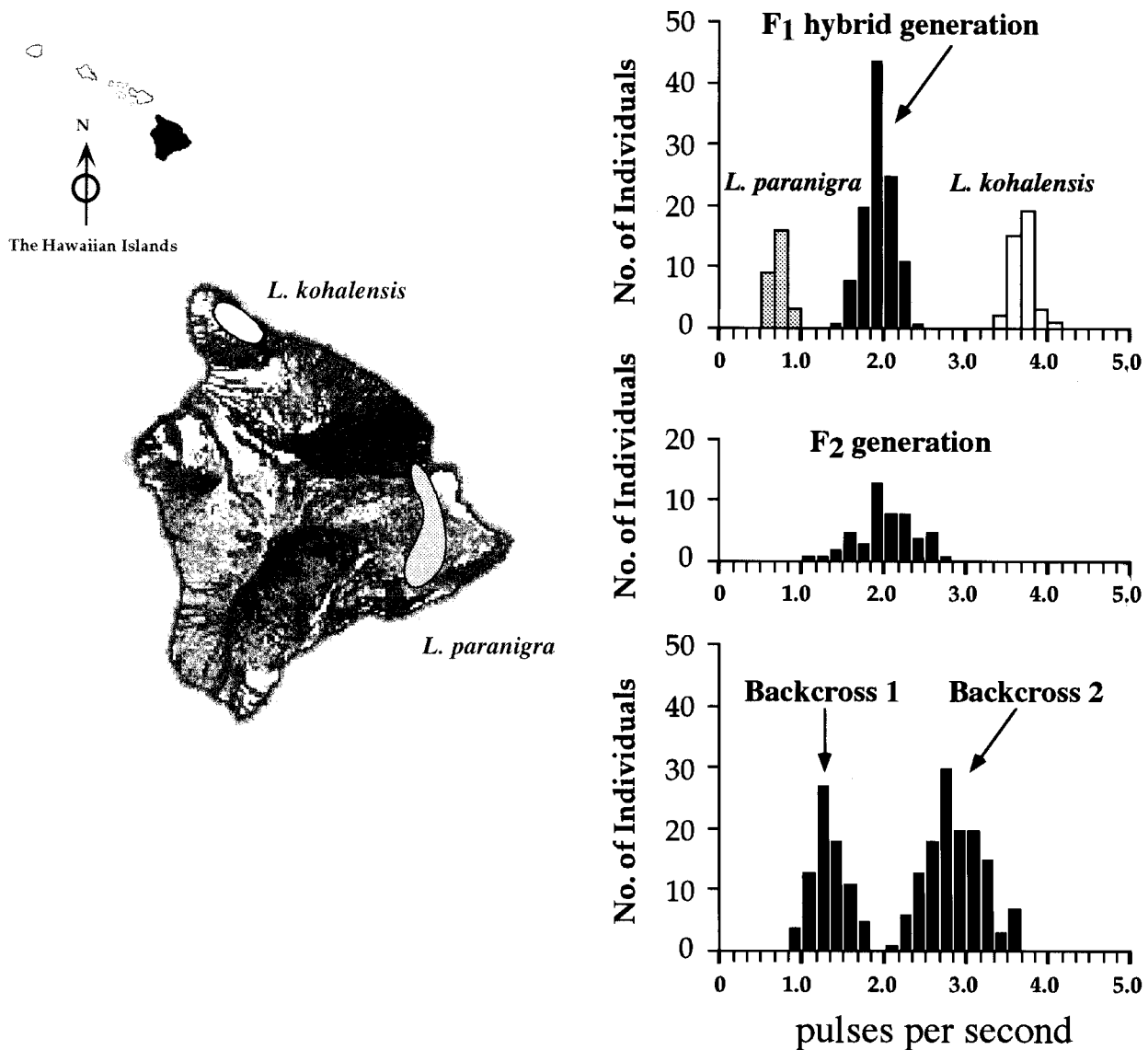


Figure 2: Distributions of *Laupala paranigra* and *Laupala kohalensis* endemic to the island of Hawaii and histograms of song pulse rate in parental, F₁, F₂, and backcross populations. Data from Shaw (1996a).

that ancestors of the current species also expressed unimodal preference functions and that changes in acoustic communication signals occurred through shifts in mean pulse rates and pulse rate preferences among populations.

Case for Many Genes of Small Effect

Four lines of evidence suggest that song phenotypes in *Laupala* change through the modification of many genes of small effect. First, the distribution of character states (pulse rate and acoustic preference) among different spe-

cies of *Laupala* represent a quantitative range of song variants (Otte 1994; Shaw 2000a; Shaw and Herlihy 2000). While intraspecific range of pulse rate is narrow, the mean pulse rates of different species occur across a range from 0.5 to 4.5 pulses per second. Thus, song states in the genus are continuous. Second, inheritance patterns of pulse rate and acoustic preference in *L. paranigra* and *L. kohalensis* hybrids appear additive (Shaw 1996a, 2000b). Third, a classic quantitative genetic study of song (Shaw 1996a) suggests effects of small magnitude on the X chromosome. Reciprocal crosses between *L. paranigra* and *L. kohalensis*

reveal relatively small, but statistically significant, pulse rate differences between F_1 males (males are XO in *Laupala*). Finally, segregation patterns of second-generation hybrids are consistent with eight genetic factors estimated by biometrical methods (Shaw 1996a). This value likely reflects the number of freely segregating units (in *Laupala*, $n=8$ chromosomes) and hence an underestimate (Zeng et al. 1990). We know less about the patterns of inheritance that underlie female acoustic preference. Current data suggest additivity in hybrid generations and segregation in backcross generations and therefore a similar genetic architecture to male song differences (Shaw 2000b). Future study aims to provide experimental evidence for genetic patterns of inheritance of acoustic preference between the focal taxa.

While this reasoning suggests that the acoustic communication trait differences between *L. paranigra* and *L. kohalensis* are caused by many genes of small effect, drawing such conclusions from second-generation hybrid segregation patterns is questionable (Shaw 1996a; Ritchie and Phillips 1998; Via and Hawthorne 1998). Polygenic segregation patterns can also result from genes of minor effect or major effect with gene interaction, or with a mixture of directionality and magnitudes of effect (e.g., Templeton 1977; Bradshaw et al. 1995; Liu et al. 1996). A QTL approach to studying genetic architecture avoids some of the limitations of the biometrical method. The basic strategy of all QTL methods is to test the effect of allelic substitution between progeny marker classes to identify those chromosomal regions that cosegregate with phenotypic variation. A QTL mapping study of song will allow us to look more closely at the effect of specific regions of the genome on variation in pulse rate.

Directionality of Effects in the Acoustic Communication System of Laupala

Our goals are to generate sample sizes large enough to examine both the magnitude and directionality of effects of both song and preference differences that distinguish *L. paranigra* and *L. kohalensis*. We outline our approach and preliminary results below.

Quantitative trait loci can provide information on the role of selection versus genetic drift (Orr 1998) because directional phenotypic evolution by natural or sexual selection should result in the substitution of alleles at QTL that further increase (or decrease) the trait under selection, in this case, song and preference behavior. A genetic drift model of change can be rejected if a majority of QTL increase pulse rate in *L. kohalensis* (the fast singer) and likewise decrease pulse rate in *L. paranigra* (the slow singer). Because the null drift model predicts a mixture of effects (table 1), such a finding would allow us to rule

out mechanisms of speciation with a significant drift component.

Material and Methods

Interspecific Hybrids

We have been studying segregation in *Laupala paranigra* and *Laupala kohalensis* and in an F_2 hybrid population (hereafter, the "mapping population") derived from 12 crosses (including both reciprocals) between these two species (hereafter, the "parentals"). Individuals of the parental species have seven autosomal pairs; females have an additional pair of X chromosomes, and males are XO. All F_2 individuals in our mapping population are male. The pulse rate phenotypes of the two parental species differ by approximately 25 standard deviations. We therefore expect that most, if not all, loci responsible for the pulse rate difference will show diagnostic differences between these two species. Seventy-two F_2 individuals were generated by pairwise full-sib intercrossing of F_1 hybrid progeny. Mature males were recorded individually with a Marantz PMD-430 portable cassette recorder. Songs were digitized using the Soundscope digitizing technology (GWI Instruments, Cambridge, Mass.). Figure 2 shows the segregating pulse rate phenotypes (for data and detailed methods, see Shaw 1996a).

Collection of Dominant (Autosomal) AFLP Markers

We have screened our mapping population for diagnostic amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995). The AFLP technique assays single base pair substitutions and length variation, generating large numbers of highly reproducible, species-specific markers (e.g., Hill et al. 1996; Paul et al. 1997). Our use of F_2 progeny allows us to identify both *L. kohalensis* and *L. paranigra* specific markers in the same individual, which enables the recovery of both parental homozygotes in the same segregating population.

Detailed methods of AFLP screening are reported elsewhere (Y. M. Parsons and K. L. Shaw, unpublished manuscript). Briefly, genomic DNA was isolated from the 72 F_2 hybrids and 20 individuals per parental species. Samples were digested with either an *EcoRI/MseI* or *EcoRI/PstI* restriction enzyme combination, and oligonucleotide adapters were ligated to the resulting fragments (primer and adapter sequences from Vos et al. 1995). Preselective (one-base) and selective (three-base) polymerase chain reaction (PCR) reduced the number of amplified fragments, which enables separation by 4% polyacrylamide gel electrophoresis (PAGE) and visualization through silver staining. Pooled parental samples ($n = 20$) together with a subset

of F_2 hybrids ($n = 12$) were screened with many combinations of primer pairs to identify suitable markers. Data on diagnostic markers (present in one parental species and absent in the other) that displayed a ratio of 3 : 1 in the mapping population (the expected ratio given Mendelian inheritance of a dominant autosomal marker) were collected for analyses. Segregation ratios were evaluated at a significance level of $\alpha = 0.05$ by a χ^2 goodness-of-fit test.

Collection of Putative Hemizygous AFLP Markers

Data on diagnostic markers that displayed a ratio of 1 : 1 in the mapping population (the expected ratio given Mendelian inheritance of a sex-linked marker) were used in putative X chromosome linkage analyses. Segregation ratios were evaluated by χ^2 goodness-of-fit tests, as discussed above.

Collection of Codominant AFLP Markers

The AFLP markers are generally dominant. However, we have identified 19 species-specific length variants, which suggests interspecies allelic status of the bands of alternate length. Seventeen of these loci appear to be autosomal, and two appear to be X linked. Confirmation of allelic identity of species-specific length variants was achieved by excising length variants from the acrylamide gel, reamplifying and sequencing the target bands.

Results and Data Analysis

Because most of our information about recombination comes from dominant markers, linkages were analyzed separately in *Laupala kohalensis* and *Laupala paranigra*, which resulted in two individual species maps. Pairwise maximum likelihood analysis (MAPMAKER/Exp 3.00;

Lincoln et al. 1992; Y. M. Parsons and K. L. Shaw, unpublished manuscript) was employed to estimate the amount of recombination between genetic loci within each species. Markers were placed into linkage groups at a log odds (LOD) threshold of 3.5 and a maximum distance of 30 cM. The linkage maps of the autosomes comprise 146 markers in 13 and nine linkage groups specific to the *L. kohalensis* and *L. paranigra* parentals, respectively (table 2). From the mapping location of 15 codominant markers, we were able to align six species-specific linkage groups, identifying these as homologous linkage groups between the two species.

Mapping analyses using the 1 : 1 segregation data resulted in 10 additional linkage groups (five per species). Two of these groups were large, one containing 16 *L. kohalensis* markers and the other containing 14 *L. paranigra* markers, which suggests putative X linkages. Two pairs of AFLP markers that mapped to these putative X linkage groups showed a complementary banding pattern in F_2 individuals. Sequencing of one pair of complementary markers confirmed that they represent the same locus, one originating from *L. paranigra* and the other from *L. kohalensis*, and that these two X linkage groups are homologous. The mapping analyses of the 1 : 1 segregation data also resulted in four additional smaller linkage groups per species (with five or fewer markers per group). These additional linkages may be spurious or may represent portions of the X chromosome that have yet to join with the main linkage group due to a lack of intervening markers.

Two kinds of analyses are discussed in this article. First, we examined the marker data in the F_2 population to determine if overall marker constitution per individual was predictive of pulse rate. The band profile for each F_2 individual was quantified separately for *L. paranigra* autosomal dominant markers, *L. kohalensis* autosomal dominant markers, codominant markers, and hemizygous markers. For each dominant AFLP marker locus, an F_2 individual was given a value of 1 if it showed a band

Table 2: The current state of the *Laupala* linkage map

Map feature	Total	<i>Laupala kohalensis</i>	<i>Laupala paranigra</i>
		in map	in map
Codominant markers (1 : 2 : 1, autosomal)	19	17	16
Dominant markers (3 : 1, autosomal)	176	68	62
Linkage group total (autosomal)	...	13	9
Autosomal map size (cM)	...	995.0	801.8
Mean distance between autosomal markers (cM)	...	11.8	10.4
Hemizygous markers (1 : 1, putative X)	122	16 (30)	14 (25)
Putative X map size (cM)	...	131.7 (275.0)	107.5 (204.4)
Mean distance between putative X markers (cM)	...	8.2 (9.2)	7.7 (8.2)
Homologous linkage groups	7	7	7

Note: For hemizygous markers, data before parentheses are for the largest linkage groups only; data in parentheses include all linkage groups containing 1 : 1 segregating markers.

present and a value of 0 if it showed a band absent (hence, for each marker locus, $M/M = 1$, $M/- = 1$, $-/- = 0$, where $M =$ band present and $- =$ band absent). For codominant markers, where heterozygotes could be determined, $M/M = 2$, $M/m = 1$, and $m/m = 0$ (*L. kohalensis* alleles for codominant loci were arbitrarily given a value of 1 and *L. paranigra* alleles a value of 0). For hemizygous markers, $M/O = 1$ and $-/O = 0$ (for *L. kohalensis* specific bands) and $m/O = 0$ and $-/O = 1$ (for *L. paranigra* specific bands), where X/O refers to the hemizygous genotype. For each of the four quantifications above, an

individual's total marker tally was calculated by summing across their entire display of bands for all primer pair combinations, and the correlation between total marker score and pulse rate was calculated in our F_2 mapping population (results are presented in fig. 3). Overall, F_2 individuals with a higher proportion of *L. kohalensis* markers tend to sing with a faster pulse rate, and individuals with a higher proportion of *L. paranigra* markers tend to sing with a slower pulse rate. All relationships were significant by Spearman rank correlation (fig. 3).

Second, we conducted a preliminary QTL analysis of

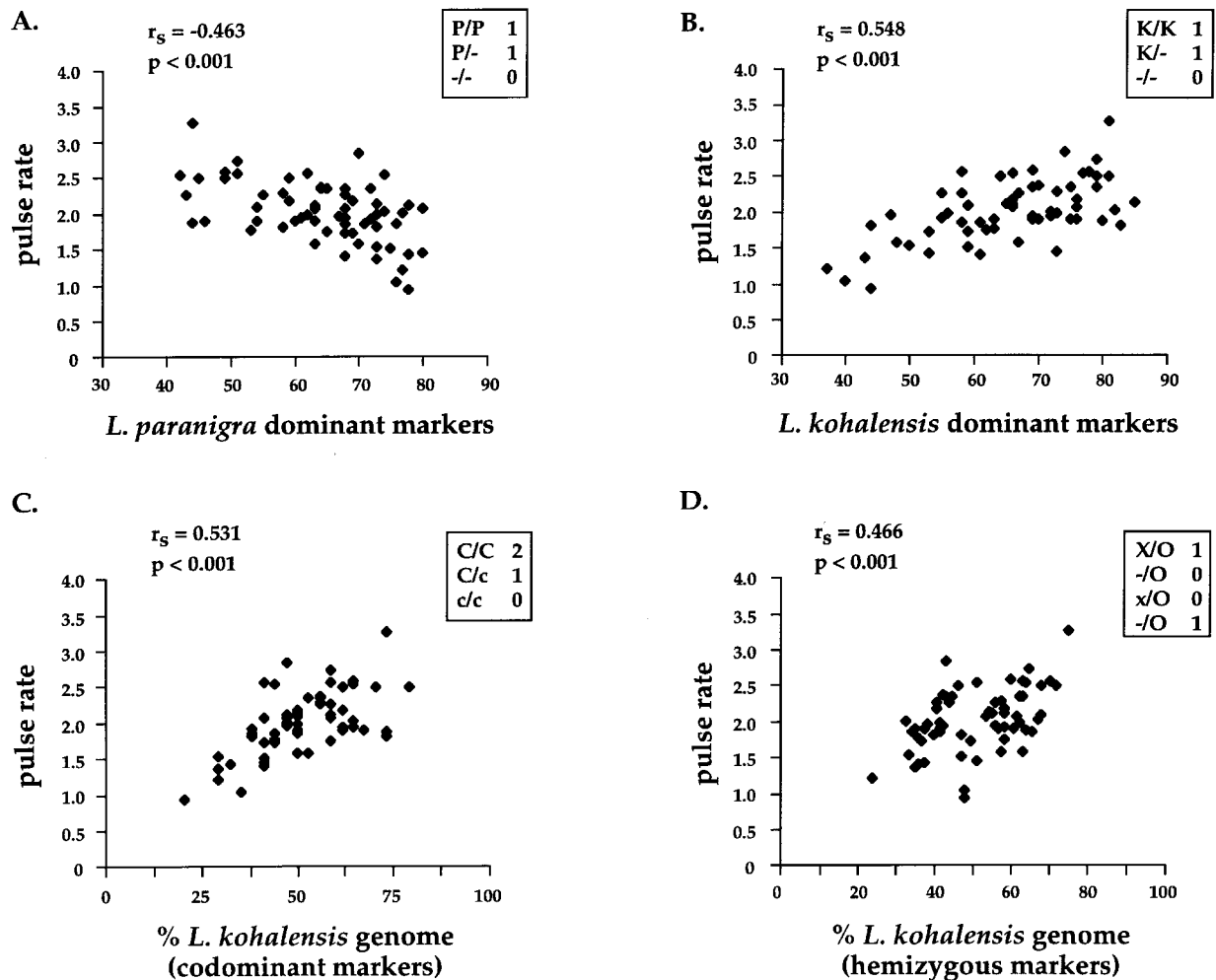


Figure 3: The relationship in the F_2 mapping population between pulse rate and marker score. A, Dominant marker score and pulse rate for *Laupala paranigra* markers. B, Dominant marker score and pulse rate for *Laupala kohalensis* markers. C, Codominant marker score and pulse rate. D, Hemizygous marker score and pulse rate. For each dominant marker locus, an F_2 individual showing a band present was given a score of 1 and was given a score of 0 for band absence (i.e., for each marker locus, $M/M = 1$, $M/- = 1$, $-/- = 0$, where $M =$ band present and $- =$ band absent). For each codominant marker locus, an F_2 individual was given a 2 (if showing two *L. kohalensis* [M/M] alleles), a 0 (if showing two *L. paranigra* [m/m] alleles), or a 1 (if showing one of each parental [M/m] allele). For each hemizygous marker locus, $M/O = 1$, $-/O = 0$, $m/O = 0$, and $-/O = 1$, where X/O refers to the hemizygous genotype, M to *L. kohalensis* alleles, and m to *L. paranigra* alleles. An individual's total marker tally for each class of markers was calculated by summing across its entire display of bands for all primer pair combinations scored.

song variation using the interval mapping method in QTL Cartographer (Basten et al. 1994–2000). We obtained significant results, identifying 12 putative QTL, including four at *L. paranigra* specific, five at *L. kohalensis* specific, and three at common map localities (designated by codominant markers). Threshold significance values were determined empirically using the permutation approach of Churchill and Doerge (1994).

The significant marker-phenotype associations found at the three codominant marker map locations in both species' analyses add corroborative strength to the conclusion of QTL in these regions. We examined each of these codominant marker associations separately. Significant one-way ANOVA (in each case, $P < 0.001$) confirmed that there is significant heterogeneity in pulse rate among genotypic classes. In all three cases, Tukey's method for pairwise comparisons revealed that the mean heterozygote phenotypes were significantly different from the mean phenotype of one homozygote class but not the other, which provides statistical support for dominance. Which homozygote-heterozygote class differed depended on the locus considered (fig. 4). Table 3 summarizes these results.

Table 3 also reports the magnitude of effect for each allele as the contribution of the QTL to the difference between the two pure species' phenotypes. For each of the three codominant QTL, the magnitude of effect was calculated as half the difference between the two homozygote genotypic classes, divided by the total phenotypic difference between the species (Falconer 1989, chap. 7). The three QTL show similar magnitudes of effect, between 6% and 9%.

Discussion

The speciation models discussed in this article differ with respect to the evolutionary pathways mate recognition phenotypes are expected to take and, therefore, the anticipated directionality of effects of alleles substituted during divergence. Under the Fisher process, male and female phenotypes change in a coordinated fashion, most likely in small increments. Directional selection for higher (or lower) trait values favors divergent allelic effects that increase (or decrease) male and female phenotypes. Under the Fisher process, the evolutionary response of the male trait to sexual selection eventually is halted by the opposing force of natural selection. We would expect these patterns of genetic architecture to occur if stabilizing selection develops gradually as a result of the elimination of the tails of the trait distribution. Some more recent polygenic models of sexual selection (e.g., Schluter and Price 1993; Turner

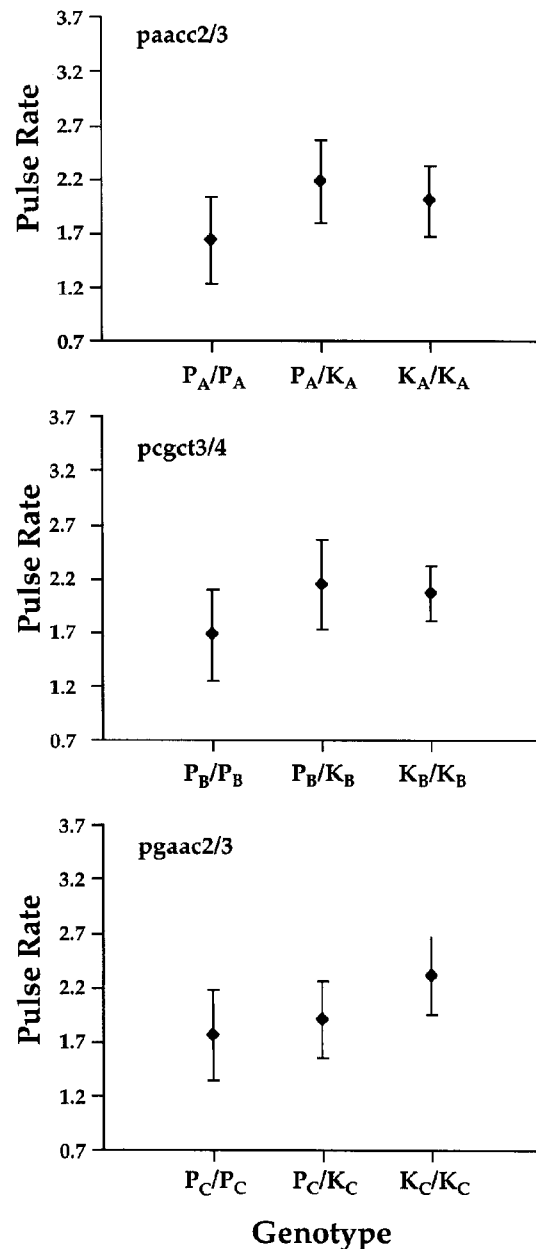


Figure 4: Mean pulse rate for each genotype of three codominant marker loci (subscripts A, B, and C) significantly associated with QTL for pulse rate. Plots show mean pulse rate + 1 standard deviation for paacc2/3, pcgct3/4, and pgaac2/3. P denotes an allele from *Laupala paranigra*; K denotes an allele from *Laupala kohalensis*. K alleles from paacc2/3 (A) and pcgct3/4 (B) are dominant to P alleles; the P allele from pgaac2/3 (C) is dominant to the K allele.

and Burrows 1995; Hall et al. 1999; Gavrillets 2000) would give qualitatively similar results with respect to directional allelic effects, although others may not (e.g., Iwasa and Pomiankowski 1995).

Table 3: Codominant markers significantly associated with pulse rate variation in *Laupala*

Marker	ANOVA	Dominance	Dominant allele	Directionality of effect	Magnitude of effect (%)
paacc2/3	$F = 11.23$, $df = 2, 60$, $P < .001$	Yes	<i>Laupala kohalensis</i>	+	6.1
pcgct3/4	$F = 7.99$, $df = 2, 60$, $P = .001$	Yes	<i>L. kohalensis</i>	+	6.5
pgaac2/3	$F = 11.53$, $df = 2, 60$, $P < .001$	Yes	<i>Laupala paranigra</i>	-	9.0

Note: All three markers showed a pattern of dominance in the F_2 mapping population. The dominant allele was not from the same species in all cases.

Other processes of divergence discussed in this article affecting mate recognition systems combine drift with selection (Kaneshiro 1976; Templeton 1980; Lande 1981; Carson 1982). During the drift phase, male and female sides of the system may become uncoupled, and the expected effects of substituted alleles in each diverging lineage will show a mix of divergent and convergent directionality. In addition, in the case of founder models, the decoupled phase occurs during a period of “disorganization,” and with relaxed selection, systems are not constrained to evolve in small increments.

We employ a QTL approach to study the genetic architecture of acoustic communication behaviors in the cricket genus *Laupala* in order to understand the pathway of evolution this component of a mate recognition system has taken. These phenotypes appear to be intimately involved in speciation and to diverge rapidly (Otte 1994; Shaw 2000a). The exceptional phenotypic difference between *Laupala paranigra* and *Laupala kohalensis*, the two focal species of this study, makes an interspecific hybrid design and QTL study extremely powerful.

Current biometrical evidence from *Laupala* suggests that male and female acoustic phenotypes evolve in a coordinated fashion and that many genes of small effect contribute to the species differences in male, and possibly in female, acoustic behaviors. Our analysis quantifying parental genetic marker proportions as a function of pulse rate in our F_2 mapping population corroborate the hypothesis that interspecific song variation has a significant, quantitative genetic basis (fig. 3). The preliminary QTL results in our F_2 mapping population add further support to a quantitative hypothesis, revealing 12 QTL in three major linkage groups. A closer examination of the codominant markers that are significantly associated with QTL suggests that the directionality and magnitude of effects of these QTL are consistent with a directional mode of selection on alleles of small effect (fig. 4; table 3).

Our future work will comprise a much larger F_2 population for several reasons. Simulation studies show that all methods have little power to detect QTL of small effect (<5%) unless a sample size of 500 or more is used, even when the heritability of the trait is high (Van Ooijen 1992; Carbonell et al. 1993; Beavis 1998). Linked QTL may be

difficult to detect with low sample sizes and may elevate the estimation of QTL effects. Moreover, simulation studies show (Beavis 1998; Lynch and Walsh 1998) that small sample sizes can overestimate QTL effects. Experimental results support this claim (reviewed in Beavis 1998). Studies with a progeny size of 100 to 400 have identified relatively small numbers of QTL of moderate to large effect (Knott et al. 1997, 1998; Li et al. 1997; Beavis 1998; Cordell et al. 1998), while a study employing a moderate population of mice (535 F_2 progeny) has identified larger numbers of QTL, which explains 1% to 16% of the total phenotypic variance (for growth and morphological traits; Cheverud et al. 1996; Leamy et al. 1998). This situation makes our hypothesis of many genes of small effect conservative since we are more likely to identify QTL of large effect if they exist. Our estimation of QTL magnitudes will likely decrease with larger sample sizes. Given our current sample size, our conclusion of small effect is surprising but not likely to change.

While the results based on our small sample size are consistent with a Fisher process, we cannot yet rule out other processes. Detecting large numbers of QTL will increase the power of any test aimed at distinguishing a history of directional selection from one of drift. A larger sample size should increase the number of detectable QTL underlying the song difference, which would allow for a statistical analysis such as proposed by Orr (1998). Following an early, short-lived drift phase in each of the alternatives to the Fisher process, selection comes to play a role in bringing the mate recognition system back into coupling. It remains unclear whether the formal method proposed by Orr (1998) will allow the rejection of anything other than a pure drift model, and alternative statistical tests may be needed.

The models discussed above target male and female sides of the mate recognition system differently, and thus the expected directionality of effect of male and female alleles will differ (table 1). This difference provides a means to separate the various models incorporating a drift component. Ultimately, to distinguish between mechanisms of speciation involving sexual selection, or a combination of selection and genetic drift, contrasts between the genetic architectures underlying male and female traits are re-

quired. Our goal is to carry out QTL studies of acoustic preference on segregating populations of females to complement our studies with males. In addition to examining basic questions of directionality and magnitude of effect of QTL underlying acoustic preference, we hope to investigate the hypothesis that the same genomic regions affecting male song also affect female preference behavior. Coordinated evolution in male/female signaling systems should be facilitated by the potential for rapid physical linkage of coordinated mutations, as appreciated by Alexander (1962).

Historical Inference

Experimental hybridization studies between species imbed the alleles of one species into the background of a hybrid or pure genotype of the other species, which facilitates the measurement of the directionality of effect of the substituted alleles. While many QTL studies are primarily focused on such phenotypic effects, historical tests use experimental hybridization designs to generate ancestral genotypes in order to “resurrect” the conditions under which new alleles have been substituted. Thus, hybridization designs can provide a means to examine the behavior of alleles in ancestral genetic backgrounds, the assumed genetic conditions under which those alleles were substituted into the population. It follows that, when test-

ing the hypothesis that a trait difference evolved by chance, we implicitly do so in a comparative, or phylogenetic, context. Many factors may influence our power of historical inference. We consider two issues: the effect of dominance and whether sister species comparisons are necessary.

Dominance

Consider two species that differ in a divergently selected trait currently used in courtship by the males of both species. Using a two-locus model, we illustrate three ways this difference might come about (fig. 5). Allelic substitution and character evolution from a most recent common ancestor along both branches leading to species 1 and 2 may have occurred (fig. 5A). Alternatively, the most recent common ancestor of species 1 and 2 may have had the genotype and phenotype of species 1 (fig. 5B), all character change having occurred along the branch leading to species 2 (or vice versa, in fig. 5C). Thus, the ancestral population may have been genotypically intermediate or more similar to one or the other parent. In all cases, as allele substitutions occur, both heterozygous and homozygous genotypes arise in the transitional populations from a common ancestor to the extant species. As allele substitutions occur under either additive or dominant relationship to existing alleles, phenotypes become visible to

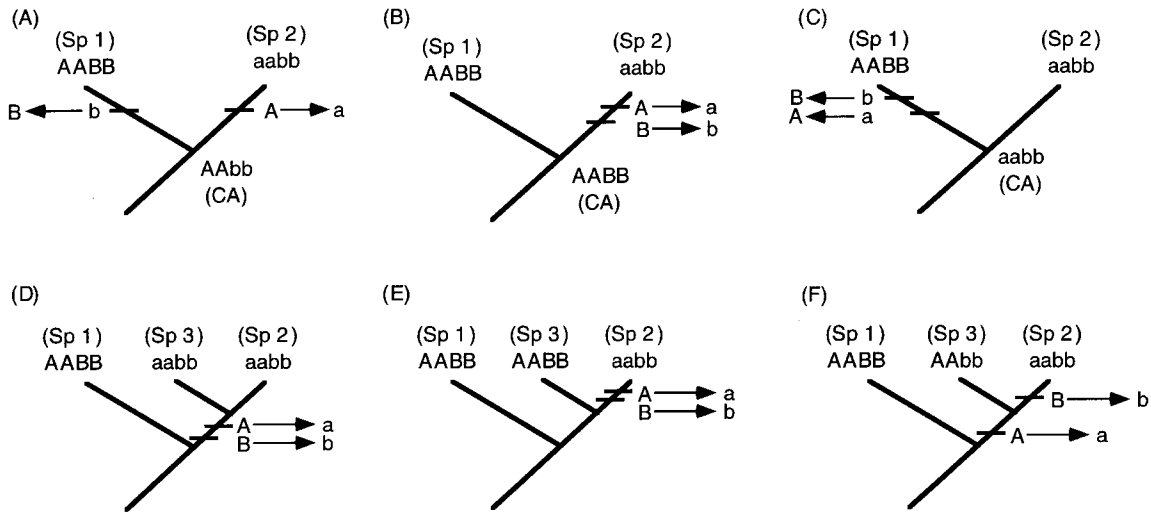


Figure 5: Inferring directional selection from QTL directionality of effects in a phylogenetic context. A courtship trait difference between species (*Sp*) 1 and 2 is controlled by two loci, *A* and *B*, as illustrated. Alleles *a* and *b* decrease and alleles *A* and *B* increase the trait value. In A–C we illustrate three ways this difference might come about, and this depends on the genotype of the common ancestor (*CA*). Allelic substitution may occur along branches leading to both species 1 and 2 (A) or exclusively to species 2 (B) or exclusively to species 1 (C). Other possible genotypic intermediates of the *CA* do not alter the conclusions and therefore are not shown. In D–F, a third species bisects the branch descending to species 2 and 3. Depending on the genotype of species 3, its close relationship to species 2 can help resolve the branch location of allelic substitution that distinguishes species 1 and 2.

directional selection in either the heterozygous or homozygous condition, respectively. Thus, dominance between alleles should not invalidate the biased directionality of effects argument under directional selection.

Our significant QTL associated with codominant markers (fig. 4) suggests a dominance interaction between QTL alleles from *L. paranigra* and *L. kohalensis*. We observed dominance of the *L. kohalensis* alleles in two of the three significant marker loci and dominance of the *L. paranigra* allele at the third significant marker locus. An observation that the most recent common ancestor was genotypically like *L. kohalensis* at the first two QTL and like *L. paranigra* at the third would be compatible with the general rule that most allelic substitutions are recessive (reviewed in Orr 1991). In other words, the rule for allelic substitutions leads us to predict that the dominant alleles are the ancestral alleles. Figure 5A, therefore, illustrates our prediction of the ancestral genotype for one *L. paranigra* locus and one of the *L. kohalensis* loci. We generally cannot know the ancestral genotype with certainty. However, in closely related species groups, outgroup comparisons identified using phylogeny may allow us to test this hypothesis. Comparative studies of outgroup species should be fruitful in *Laupala* because several other species appear to be extremely closely related to *L. paranigra* and *L. kohalensis*.

Are Sister Species Comparisons Necessary?

Comparative studies of ingroup species may also be fruitful. Relatively speaking, sister species comparisons will enhance the probability that the genetic backgrounds created in hybridization experiments are valid for historical tests of drift versus selection. This follows because the masking of initial genetic changes accompanying speciation by later genetic changes will be minimized by studying sister species. However, the critical feature of accurate historical hypothesis testing in a hybridization study is recency of descent rather than sister species relationship per se. Paradoxically, closely related nonsister species may be useful, and even preferable, for some questions provided that the genetic backgrounds created by hybridizing nonsister species are adequate for historical hypothesis testing.

Figure 5D–5F shows a third species that bisects a branch descending to one of two focal species (species 1 and 2). Species 3 may provide a historical sample useful for resolving the branch location of phenotypic and genotypic evolution that distinguishes species 1 and 2 if the genetic content underlying a quantitative trait at the branching event to species 3 is preserved in present-day populations. Hybridization studies under this form of historical relationship will not alter the basic power of inference but, rather, will enhance our ability to resolve when changes

occurred and more accurately to determine the causal basis of speciation.

Conclusion

An important goal in evolutionary biology is to explain the evolutionary forces that cause new species to form. If Darwin's (1879) observation that newly diverged lineages frequently differ in sexual characters holds true, major research effort should focus on the mechanisms that cause sexual competition, intersexual signaling, and courtship coordination phenotypes to change. A genetic dissection of these traits may provide insight into how evolutionary shifts occur, as forecast by Mather (1966, p. 334): "The genetical architecture of a character and the kind of variation to which it is subject ... reflect the kind of selection to which the character has been subject in the past."

Our knowledge of the genetic basis of premating isolation currently lags behind that of postmating isolation (Hollocher 1998; Ritchie and Phillips 1998), yet the evolution of premating isolation may be the primary cause of speciation in many taxa (Coyne et al. 1994), including *Laupala* (Shaw 1996a, 1996b). Genetic and phenotypic differences between closely related species that can be examined through experimental hybridization studies will potentially provide information about the evolutionary paths a mate recognition system can take and, ultimately, the mechanism of species differentiation through premating isolation. The genetic basis of hybrid sterility and inviability between species of *Drosophila* suggests that many genes are often involved and interactions among genes are common (reviewed in Wu and Palopoli 1994). Too few studies have been conducted in sufficient detail to provide generalities regarding "rules" for the genetics of premating reproductive barriers (Ritchie and Phillips 1998; Shaw 2000b). Many genes may be involved (as in incipient *Drosophila* species; Wu and Hollocher 1998). Focused studies of reproductively important phenotypes (e.g., male genitalia and cuticular hydrocarbons; Coyne et al. 1994; True et al. 1997) have found fewer major gene effects, however.

We have evidence for several genes of small effect underlying the acoustic signaling changes in the early divergence of *Laupala*. Evidence from other studies of acoustic communication evolution (Hoy and Paul 1973; Hoy 1974; Hoy et al. 1977; Butlin 1993, 1996; Ritchie 2000) suggests that a polygenic mode of divergence will typify the genetic basis of premating isolation by acoustic divergence. Other aspects of genetic architecture, such as the directionality of allelic effects, may inform us about the causal mechanisms governing this divergence.

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