Mapping Unexplored Genomes: A Genetic Linkage Map of the Hawaiian Cricket Laupala

Y. M. Parsons[1] and K. L. Shaw[2]

Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138

Manuscript received December 10, 2001
Accepted for publication August 16, 2002

ABSTRACT

As with many organisms of evolutionary interest, the Hawaiian cricket Laupala genome is not well characterized genetically. Mapping such an unexplored genome therefore presents challenges not often faced in model genetic organisms and not well covered in the literature. We discuss the evolutionary merits of Laupala as a model for speciation studies involving prezygotic change, our choice of marker system for detecting genetic variation, and the initial genetic expectations pertaining to the construction of any unknown genomic map in general and to the Laupala linkage map construction in particular. We used the technique of amplified fragment length polymorphism (AFLP) to develop a linkage map of Laupala. We utilized both *Eco*RI/*Mse*I- and *Eco*RI/*Pst*I-digested genomic DNA to generate AFLP bands and identified 309 markers that segregated among F_2 interspecific hybrid individuals. The map is composed of 231 markers distributed over 11 and 7 species-specific autosomal groups together with a number of putative X chromosome linkage groups. The integration of codominant markers enabled the identification of five homologous linkage groups corresponding to five of the seven autosomal chromosomal pairs found in Laupala.

GOOD model organisms for assessing the role of prezygotic changes in speciation are often not well characterized genetically (*e.g.*, Gregory and Howard 1994; Shaw 1996a; Wells and Henry 1998; Via 1999; Rundle *et al.* 2000). Fortunately, genetic linkage maps can now be developed for virtually any genome, due to recent advances in molecular and statistical methods (Tanksley 1993; Via and Hawthorne 1998). One obvious advantage to this versatility is that linkage maps can be applied to the study of a diversity of organisms that might serve as powerful models for understanding the processes of speciation. Estimation of the numbers and effects of genes underlying trait variation between natural populations has long been of interest to evolutionary biologists (reviewed in Barton and Turelli 1989), a pursuit recently made tractable through quantitative trait locus (QTL) mapping. Applying QTL mapping technology to the genetics of speciation will be particularly powerful because different focal species are often characterized by distinct differences in functional quantitative phenotypes.

The majority of mapping studies investigating speciation questions have been conducted on organisms for which prior genomic information was available (*e.g.*, True *et al.* 1997; Kim and Rieseberg 1999). While we have learned a great deal about postzygotic incompatibility between species from such systems, our understanding of the genetic basis of prezygotic incompatibility remains extremely limited (Wu and Palopoli 1994; Coyne and Orr 1998; Ritchie and Phillips 1998). Yet many speciation processes may involve or even be caused by evolutionary changes in phenotypes expressed prior to zygote formation. Understanding the role of prezygotic traits in reproductive incompatibility would improve many speciation models (Lande 1981; Ritchie and Phillips 1998; Servedio 2000) and lead to more powerful tests of speciation hypotheses (Shaw and Parsons 2002). Linkage map technology is making this avenue of investigation possible.

Issues relevant to mapping a novel or largely unknown genome have not been well covered. Accordingly, in the present work we highlight issues to be faced when mapping an unexplored genome and illustrate these issues through our linkage map study of the Hawaiian cricket Laupala, a model for the study of speciation through the evolution of premating behavior.

The Hawaiian genus Laupala is a morphologically cryptic group of small flightless crickets endemic to the Hawaiian archipelago. Closely related species of Laupala have distinct songs and acoustic preferences (Otte 1994; Shaw 1996a, 2000; Parsons and Shaw 2001) that act as a premating behavioral system thought to confer ethological isolation between species. In all species of Laupala, the male song structure is simple, consisting of a rhythmic train of pulses produced during courtship by stridulation of the forewings. Females respond by walking toward the source of the sound to locate potential mates. Only one temporal parameter of the song, pulse rate, consistently distinguishes closest relatives within

[1]*Corresponding author:* Department of Genetics, La Trobe University, Victoria 3086, Australia. E-mail: Y.Parsons@latrobe.edu.au

[2]*Present address:* Department of Biology, University of Maryland, College Park, MD 20742.

the genus (Otte 1994). Polygenic control of pulse rate and pulse preference variation between closely related species has been demonstrated (Shaw 1996a, 2000). Thus speciation is accompanied by, perhaps even caused by, evolutionary forces acting on male song and female acoustic preference.

Laupala exhibit widely divergent acoustic variation between interfertile species and thus offer essential conditions for successful QTL mapping. Without wide phenotypic differentiation the power to identify QTL, especially those with medium-to-low magnitudes of effect, is severely curtailed. And without successful hybridization the generation of segregating populations is not possible and the power of linkage analysis is similarly reduced.

The Laupala linkage map presented here was constructed using amplified fragment length polymorphism (AFLP; Vos *et al.* 1995) as genetic markers using an F_2 intercross breeding design. We used an F_2 intercross design because it allowed the recovery of both parental recessive homozygotes in the same segregating population, in contrast to a backcross design where information on only one parent (*i.e.*, the nonrecurrent parent) is obtained. In addition, an F_2 intercross design has greater power than a backcross design because all gametes are derived from a recombinant generation (Falconer and Mckay 1997). We chose the AFLP method because genetic aspects of this insect are poorly understood. AFLP is based on restriction fragment length polymorphism (RFLP) and employs the polymerase chain reaction (PCR) to produce rapid and reproducible anonymous DNA markers for mapping purposes. Using this technique it is possible to generate large numbers of markers without any prior knowledge of the genome of interest. AFLP maps have been developed for many species of agricultural importance (*e.g.*, see Mackill *et al.* 1996; Powell *et al.* 1997; Wang *et al.* 1997; Lu *et al.* 1998; Hawthorne 2001) and, more recently, for organisms of ecological and/or evolutionary significance (Knott *et al.* 1997; Kocher *et al.* 1998; Kim and Rieseberg 1999; Naruse *et al.* 2000; Hawthorne and Via 2001).

Although genetic aspects of Laupala are poorly understood, we had certain expectations at the outset of this study. Karyotypic analysis in Laupala reveals seven autosomal pairs and a sex determination system where females are XX and males are XO (K. L. Shaw, personal communication). Thus, we expected our analyses to generate eight linkage groups. In addition, with a dominant marker system and an F_2 intercross design, we anticipated two separate parental maps. This occurs because only two marker classes (band presence in dominant homozygotes and heterozygotes and band absence in recessive homozygotes) are observed in a dominant marker system, and offspring are therefore classified as being like or unlike one parent only. Because heterozygotes cannot be distinguished from dominant homozygotes, one can detect recombination only between recessive null alleles inherited from the homozygous recessive parent. Separate maps derive from the collection of recombination frequencies between recessive markers from within either parental genome. Using an F_2 intercross design with a dominant marker system essentially provides two parental maps as would be obtained if a reciprocal backcross design were used (*e.g.*, see Chu and Howard 1998). The inclusion of codominant markers, however, facilitates the identification of homologous linkage groups between the two parental maps. The development of this linkage map will provide a foundation for speciation analysis of prezygotic changes and a future contrast to the speciation genetics of postmating isolation.

MATERIALS AND METHODS

Mapping population: Interspecific hybrids were previously generated between the closely related species *Laupala paranigra* and *L. kohalensis* from Kaiwiki and Kupehau localities on the Big Island of Hawaii where both species are endemic (see Shaw 1996a for details). Briefly, wild-caught females were allowed to oviposit and a number of species-specific lines were established. Following one generation random individuals were selected and subsequent mating of individual full-sib F_1 hybrid progeny from 12 interspecific crosses (including both reciprocals) generated 72 male F_2 offspring that constitute the mapping population. Only male offspring were utilized, as the underlying goal of this research is to identify QTL associated with the male courtship song. Reciprocal backcross hybrids (Shaw 1996a) were used for the initial AFLP assay.

DNA extraction: Whole frozen adult individuals were ground with a plastic pestle in a microcentrifuge tube in DNA extraction buffer [150 mm NaCl, 50 mm Tris-HCl (pH 7.5), 50 mm EDTA (pH 8.0), 1% SDS]. Proteinase K was added to a final concentration of 40 ng/ml prior to incubation for 1 hr at 65°. DNA was recovered in 50 μl 1× TE (10 mm Tris, 1 mm EDTA, pH 7.5) following phenol extraction and ethanol precipitation. Typical yields ranged from 20 to 50 μg DNA and samples were diluted to 250 ng/μl for AFLP analysis. An aliquot of 1 μl of each sample was gel electrophoresed to confirm DNA quality and quantity. Two pooled samples composed of the DNA of 15–20 individuals from each parental population were assembled for the AFLP assay to identify species-specific bands.

AFLP assay: Assays were conducted with both *Mse*I/*Eco*RI and *Pst*I/*Eco*RI restriction-digested DNA using adapters and primers as originally described (Zabeau and Vos 1993; Vos *et al.* 1995; and see Hawthorne 2001) following the manufacturer's protocols [GIBCO BRL (Gaithersburg, MD) AFLP starter primer kit, Life Technologies]. Additional adapters and oligonucleotide primers were obtained from Operon Technologies (Alameda, CA). PCR reactions for both preselective (primers with one additional base pair) and selective PCR (primers with three additional base pairs) were carried out in a total volume of 10 μl. Preselective reactions were diluted 1:40 and a 2-μl aliquot was used in selective PCR reactions. PCR products were separated by electrophoresis at 50 W for 2.5–3.5 hr through 5% polyacrylamide gels (Sequagel, National Diagnostics, Atlanta) using a 40 × 20-cm gel rig. Bands were visualized following silver staining (Silver Sequence staining reagents, Promega, Madison, WI) and, after overnight air drying, gels were scanned for archiving and computer-assisted visualization using Adobe Photoshop.

Genotyping: Diagnostic AFLP markers (present in one parental population and absent in the other) that displayed a

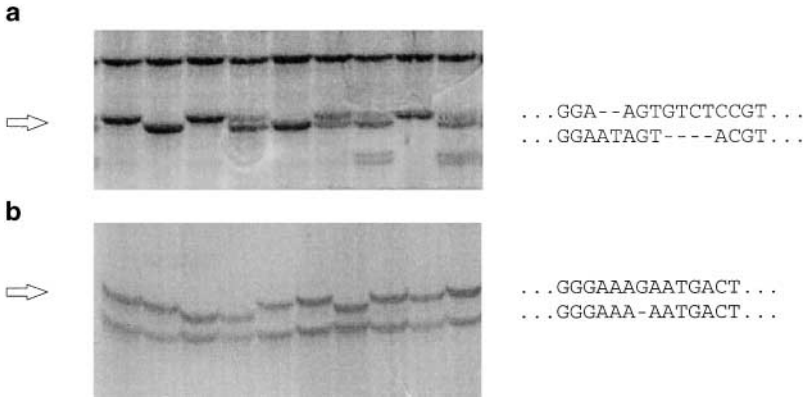

FIGURE 1.—(a) Codominant banding pattern of 3:1 segregating AFLP marker pcaac2/3 with sequence data illustrating a 4-bp allelic size variation. (b) Complementary banding pattern of 1:1 segregating AFLP marker pctcc4/5 with sequence data illustrating a 1-bp allelic size variation.

ratio of 3:1 in the mapping population (the expected ratio given Mendelian inheritance of a dominant marker) were scored for autosomal linkage analyses. In addition, markers displaying a 1:1 segregation (the expected ratio of X-linked markers given that Laupala males are haploid for the X chromosome) were scored for putative X-linkage analyses. Segregation ratios were tested using chi-square goodness of fit at a significance level of $\alpha = 0.05$. Markers were named using the following convention: "mnnnnx" or "pnnnnx," where "m" indicates the use of the *Mse*I/*Eco*RI restriction enzyme combination, "p" indicates the *Pst*I/*Eco*RI combination, "nnnn" indicates the two additional bases used in the *Mse*I or *Pst*I selective primer and the *Eco*RI selective primer, respectively, and "x" the marker number for that primer combination.

Codominant markers: AFLP markers are generally dominant, resulting in heterozygotes that cannot be distinguished from dominant homozygotes. However, segregation patterns and band intensities in the F_2 mapping population of two closely migrating bands on several gels suggested codominant inheritance. Confirmation of allelic identity of species-specific length variants was achieved by excising the relevant bands from the gel, reamplifying, and sequencing as follows. Gels were rehydrated in distilled water for 15 min; individual bands were excised and placed in 500 μl extraction buffer (0.5 M $NH_4C_2H_3O_2$) and incubated for 1 hr at 55°. The supernatant was removed to a fresh tube following centrifugation at 1400 × *g* for 15 min. DNA was recovered following ethanol precipitation overnight at 4° and resuspended in 50 μl 1× TE buffer. Reamplification was performed in a total volume of 30 μl using 3–6 μl recovered DNA solution and the relevant primer pair. Following gel extraction with Geneclean (Bio 101), purified bands were sequenced by dideoxy-terminated cycle sequencing (ABI Ready Reaction kit and ABI 373 or 3100 DNA sequencer) using the *Pst*I selective AFLP primer. Resulting sequences were assembled and compared using Sequencher computer software.

Linkage analysis: Maximum-likelihood linkage analyses were performed using MAPMAKER/Exp. Version 3.00 (LANDER *et al.* 1987). For markers segregating 3:1, band absence was coded as A for homozygote for the parental allele *a* and band presence as C for not a homozygote for parental allele *a*, when the band was absent in parent *A*. Band absence and presence were coded similarly as B and D for parent *B*. Codominant markers were coded either A or B as above or H for heterozygote. Markers that segregated 1:1 were coded and analyzed as for a F_2 backcross population. Markers were sorted into linkage groups with an initial threshold LOD score of 3.0 and a maximum genetic distance of 40 cM. For linkage groups with nine or fewer markers the most likely order within each group was determined using the "compare" command. For all other groups, the "order" command was employed to obtain the order of markers with unique placement, followed by the "try" command to find the most likely placement of the remaining markers, and subsequent orders were tested using the "ripple" command. Additional markers were placed into linkage groups at a threshold LOD score of <3.0 if the log-likelihood obtained with the "try" command was >2.0. Map distances were computed using the Kosambi mapping function that incorporates the possibility of crossover interference.

RESULTS

An initial AFLP assay was conducted to establish the presence of sufficient genomic variation for marker development between the closely related species *L. kohalensis* and *L. paranigra*. A total of 2285 bands were visualized following screening of pooled parental samples and backcross progeny with 32 *Mse*I/*Eco*RI primer-pair combinations. Polymorphism was identified in 40% of the resulting bands and 10% of these (*i.e.*, 4% of total bands) were present/absent in the parental populations and segregated 1:1 in backcross progeny. Following this assay we analyzed the F_2 mapping population with the same *Mse*I/*Eco*RI primer-pair combinations and identified 79 diagnostic AFLP markers. The ratio of suitable bands per primer combination (2.5) was lower than anticipated from results in other AFLP studies (*e.g.*, KOCHER *et al.* 1998; LU *et al.* 1998) and the use of two six-base restriction enzymes was investigated to improve marker development efficiency. A total of 230 suitable bands were subsequently identified from 61 primer-pair combinations using *Pst*I- and *Eco*RI-digested genomic DNA and the relevant adapters and primers, resulting in an increased band:primer ratio of 3.8.

Sequence examination of 10 putative codominant AFLP bands resulted in allelic confirmation in all instances. Subsequent identification of codominant markers was based on segregation pattern and band intensity (*e.g.*, see Figure 1a). A total of 17 species-specific allelic length variants were identified and incorporated into the linkage analyses. Overall, a total of 93 primer-pair combinations were assayed, resulting in the identification of 309 AFLP bands, comprising 193 autosomal and 116 putative X-linked, that could be reliably scored and conformed to the selection criteria.

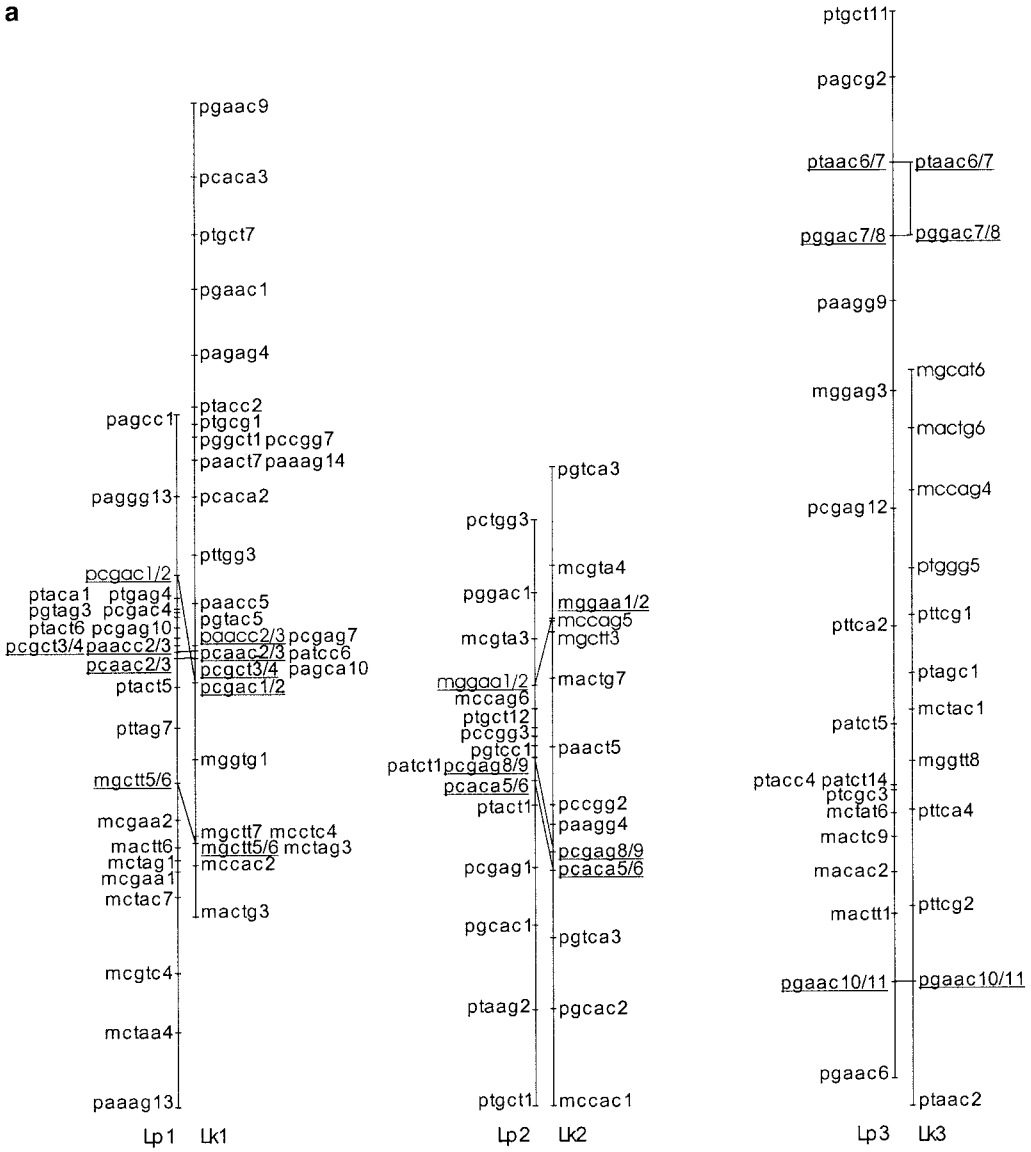

FIGURE 2.—Genetic linkage groups of *L. kohalensis* (Lk) and *L. paranigra* (Lp). (a) Autosomal linkage groups aligned via codominant markers (underlined). (b) Additional autosomal species-specific linkage groups.

Autosomal linkage groups: Of the 193 markers, 151 showing either codominant or 3:1 segregation were placed in 11 and 7 linkage groups specific to the *L. kohalensis* and *L. paranigra* parental populations, respectively (Figure 2). Five species-specific linkage groups could be aligned between the two parental populations on the basis of the map position of 15 codominant markers (Figure 2a). The number of markers per linkage group varied from 2 to 29 with map lengths ranging from 22.4 to 295 cM and an average distance between markers of 14.5 cM. The total autosomal map coverage was 1167.5 cM for the *L. kohalensis* parental population and 1021.8 cM for the *L. paranigra* parental population.

Putative X-linkage groups: Of the 116 markers exhibiting 1:1 segregation, 80 were grouped into a total of 15 linkage groups with a total coverage of 1235.9 cM (Figure 3), including 7 groups with only 2 markers each. The largest linkage group was composed of 34 markers that included two pairs of markers that appeared to represent allelic length variants of the same locus, one from each parental population. For each pair of these markers the banding pattern in the F_2 progeny was complementary, depending on which parental chromosomal region was represented in the F_2 individual (Figure 1b). Sequence examination of one pair of these complementary markers confirmed they were, indeed, length variants of the same locus. Taken together, the putative X-linkage groups covered 1235.9 cM and the average distance between markers was 15.4 cM.

DISCUSSION

To facilitate characterization of mating song variation in Laupala we developed a genetic linkage map on the basis of the recently developed technique of AFLP (Vos *et al.* 1995). Pulse rate variation of the male calling song in Laupala is a conspicuous example of a genetically based

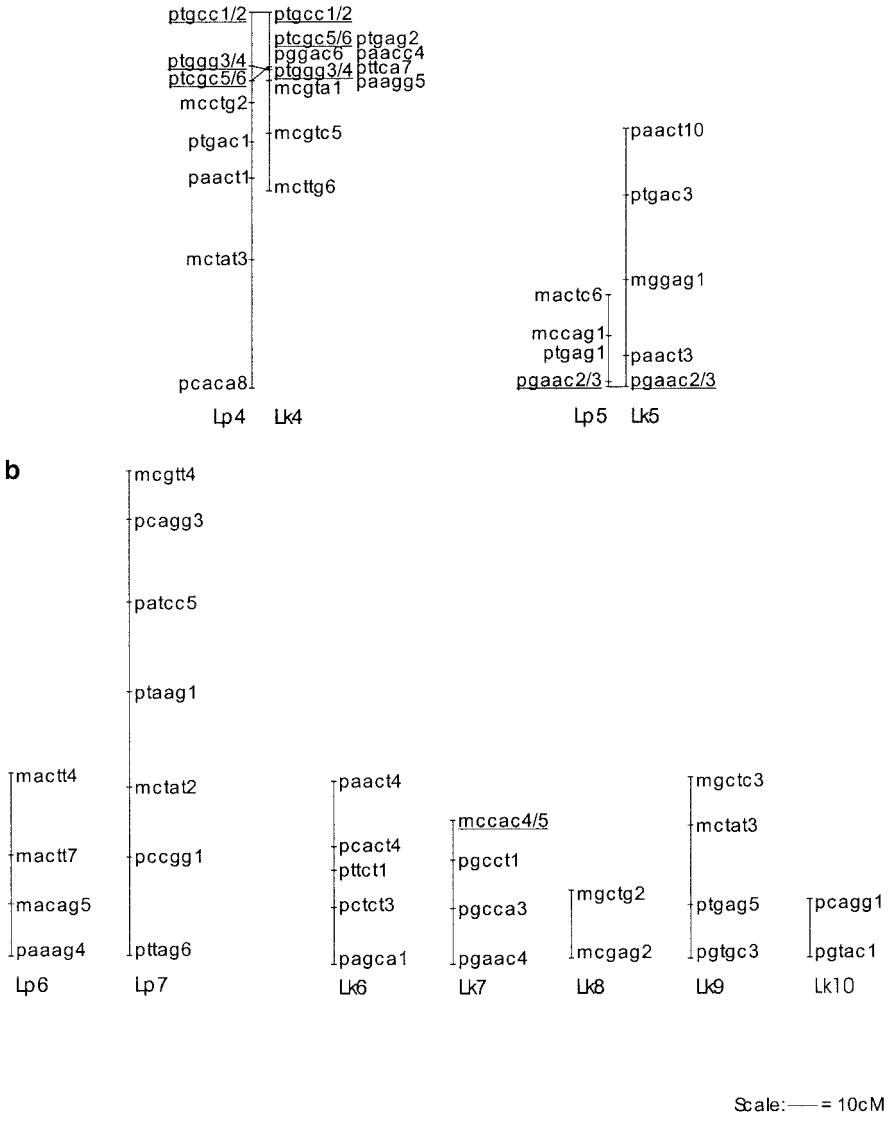

FIGURE 2.—*Continued.*

mate recognition trait, changes in which may impact the process of speciation within the genus.

The focal taxa of this study are very closely related, with both mitochondrial and nuclear sequence data displaying 0.3–0.4% sequence divergence (SHAW 1996b, 2003). Despite this close relationship the AFLP technique proved to be reliable and efficient in generating suitable mapping markers. Over 300 markers, including 17 codominant markers, were developed from 93 primer-pair combinations. The identification of several codominant markers was extremely beneficial, providing a substantial increase in linkage analysis power and allowing the identification of homologous, species-specific, autosomal linkage groups. We investigated two restriction enzyme strategies in the Laupala map construction, including the standard *Mse*I/*Eco*RI strategy (VOS *et al.* 1995) and the *Pst*I/*Eco*RI strategy described in the original AFLP methodology (ZABEAU and VOS 1993) and recently utilized in mapping of the Colorado potato beetle (HAWTHORNE 2001). Interestingly, the combined use of two six-base cutters resulted in a higher ratio of bands:primer combination. This result seems counterintuitive but could be due to an excess of AT-rich sequence in the Laupala genome (both *Mse*I and *Eco*RI contain AATT in their recognition sequence) or to the relatively large size of the Laupala genome (see below), both of which might lead to large numbers of short fragments that were subsequently not detected in our electrophoresis system. Investigators launching mapping projects of unexplored genomes of large size may benefit by choosing two six-base cutters for initial AFLP screening.

Linkage analysis resulted in the placement of 231 markers (from a total of 309) into 11 *L. kohalensis*-specific and 7 *L. paranigra*-specific autosomal linkage groups and 15 putative X groups with an average marker distance of 14.8 cM overall. Five autosomal linkage groups from each species were identified as homologous using 15 codominant AFLP markers. Laupala has a haploid chromosome content of eight (K. SHAW, unpublished

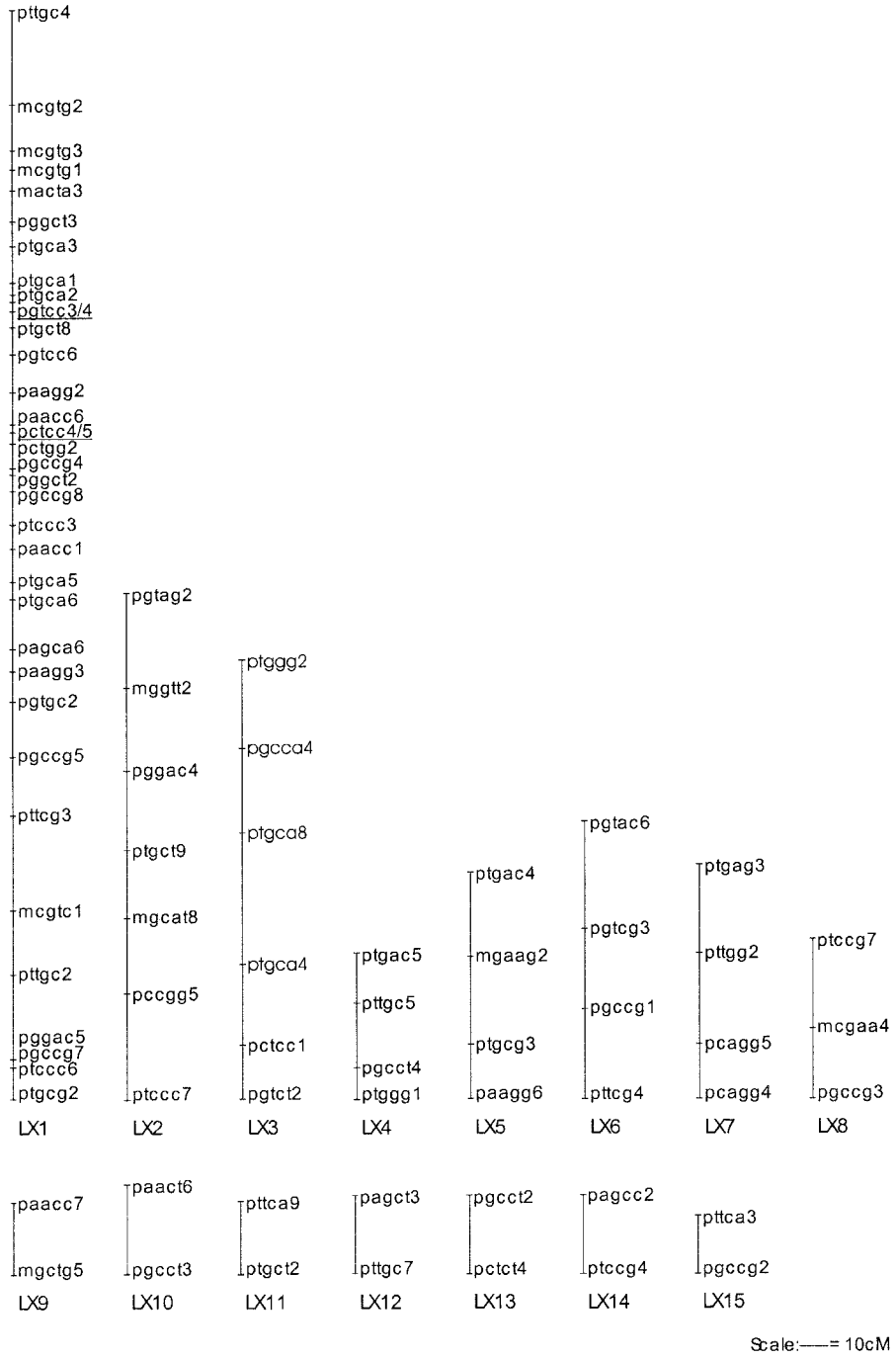

FIGURE 3.—Putative X chromosome linkage groups.

data) and it is likely that the homologous linkage groups correspond to five of seven autosomal chromosome pairs. Taking an average of the total map length obtained for each parental species together with the putative X map length, we obtained a recombinational map length of 2330 cM. The Laupala genome size has been estimated at 1900 Mb (PETROV *et al.* 2000) and the relationship between physical genome size and recombinational size, given the present results, is therefore 815 kb/cM, which falls within the range calculated in other insects (*e.g.*, see HUNT and PAGE 1995; ANTOLIN *et al.* 1996; YASUKOCHI 1998; BEEMAN and BROWN 1999).

The additional linkage groups resulting from our analysis suggest that map coverage is incomplete with some of the groups representing separate segments of the same chromosome. Spurious linkages cannot be excluded, however, and the placement of many 1:1 segregating markers into groups of only two markers is problematic and may prove to be erroneous. It is also possible that some markers segregating 1:1 are the result of segregation distortion and this possibility needs to be investigated further. Our assumption of 1:1 segregation reflecting X-linked markers is supported, however, by the inclusion of two sets of complementary markers in the largest linkage group.

AFLP markers in this study were chosen on the basis

of the presence/absence between pooled parental DNA samples. Markers generated therefore represent species-specific rather than parental-cross-specific markers and can be used for genotyping in all interspecific hybrid crosses between *L. kohalensis* and *L. paranigra*. This strategy should prove effective when the number of offspring generated between any single cross is insufficient to provide the necessary power to identify QTL of small effect (Beavis 1998). Combining offspring from several crosses has been suggested as a means of increasing the likelihood of identifying all alleles affecting a trait within a population (Xie *et al.* 1998).

In choosing a marker system for the construction of the Laupala linkage map, we considered the inherent trade-offs between resource investment and benefits of various marker systems. Codominant systems (*e.g.*, microsatellites, RFLPs) are generally more informative because (1) heterozygotes can be distinguished and (2) they can often be utilized across species. However, codominant systems also require greater initial effort and financial investment. Dominant systems (*e.g.*, RAPDs and AFLPs) are quicker, easier, and cheaper to develop but provide less information due to the heterozygote and band anonymity (Mueller and Wolfenbarger 1999). The large numbers of markers generated with dominant systems serve to offset the reduced information content, however, and simulation studies have shown that mapping with dominant markers can be efficient when combined with codominant markers (Jiang and Zeng 1997). In addition, for an unexplored genome the ability to proceed without *a priori* genetic knowledge is a distinct advantage. The extensive use of dominant markers in map construction attests to their effectiveness. Many insect maps published to date (*e.g.*, see Hunt and Page 1995; Antolin *et al.* 1996; Chu and Howard 1998; Laurent *et al.* 1998; Yasukochi 1998; Beeman and Brown 1999; Gadau *et al.* 1999) have been constructed with RAPD markers. However, the ease and accessibility of anonymous AFLP bands to sequencing is one reason why the AFLP technique is preferable to other dominant marker systems, as recently argued in a review by Mueller and Wolfenbarger (1999). As we have shown here codominant AFLP markers can also be identified providing additional mapping power. Our success with AFLP marker development in Laupala and that of other mapping studies (*e.g.*, Hawthorne 2001) provides evidence of the advantages and feasibility of mapping in uncharted genomes to researchers contemplating similar studies of lesser known organisms.

In any mapping study the marker system is of secondary importance to the segregating phenotype in hybrid progeny. QTL analyses are generally conducted in segregating populations where linkage disequilibrium is maximized. This increases the power to test the effect of allelic substitution between progeny marker classes and to identify those chromosomal regions that cosegregate with phenotypic variation. The choice of mating design is likely to be constrained or dictated by available resources, as well as by the reproductive characteristics of the organism itself. For diploid systems the choice will generally be limited to a backcross or intercross design. If a dominant marker system is used, information on heterozygotes in an intercross design will be lost due to the presence of one band masking the null allele. In backcross and haplodiploid systems this loss of information is avoided as progeny have only one informative allele. However, in the case of a backcross design the resulting map is specific to the nonrecurrent parent only. Reciprocal crosses and the subsequent typing of double the number of progeny will be required to identify QTL in both parents (*e.g.*, see Chu and Howard 1998). As we have demonstrated here an F_2 intercross design allows both parental homozygotes to be recovered in the same segregating population such that two parental-specific maps will be generated. Given that homologous linkage groups can be aligned using codominant markers, the F_2 intercross design provides a more efficient approach when genetic information from both parental genomes is sought.

With the vast array of phenotypic traits now accessible to QTL analysis the paradigm of QTL mapping needs to be expanded to include unexplored genomes. The ultimate goal of our research is to investigate the genetic architecture underlying the Laupala male calling song and we are currently in the process of generating sample sizes in segregating generations large enough to examine both the magnitude and directionality of effects of mating song that distinguish *L. paranigra* and *L. kohalensis*.

We thank Joshua Mell and Sky Lesnick for technical assistance and two anonymous reviewers for comments on an earlier draft. This research was funded by the Sloan Foundation and National Science Foundation grant no. DEB-9729325 awarded to K.L.S.

LITERATURE CITED

Antolin, M. F., C. F. Bosio, J. Cotton, W. Sweeney, M. R. Strand *et al.*, 1996 Intensive linkage mapping in a wasp (*Bracon hebetor*) and a mosquito (*Aedes aegypti*) with single-strand conformation polymorphism analysis of random amplified polymorphic DNA markers. Genetics **143:** 1727–1738.

Barton, N. H., and M. Turelli, 1989 Evolutionary quantitative genetics: How little do we know? Annu. Rev. Genet. **23:** 337–370.

Beavis, W. D., 1998 QTL analyses: power, precision, and accuracy, pp. 145–162 in *Molecular Analysis of Complex Traits*, edited by A. H. Paterson. CRC Press, Cleveland.

Beeman, R. W., and S. J. Brown, 1999 RAPD-based genetic linkage maps of *Tribolium castaneum*. Genetics **153:** 333–338.

Chu, J., and D. Howard, 1998 Genetic linkage maps of the ground crickets *Allonemobius fasciatus* and *Allonemobius socius* using RAPD and allozyme markers. Genome **41:** 841–847.

Coyne, J. A., and H. A. Orr, 1998 The evolutionary genetics of speciation. Proc. R. Soc. Lond. Ser. B Biol. Sci. **353:** 287–305.

Falconer, D. S., and T. F. C. Mckay, 1997 *Introduction to Quantitative Genetics*, Ed 4. Addison-Wesley/Longman Limited, Essex, UK.

Gadau, J., R. E. Page and J. H. Werren, 1999 Mapping of hybrid incompatibility loci in Nasonia. Genetics **153:** 1731–1741.

Gregory, P. G., and D. J. Howard, 1994 A post insemination barrier to fertilization isolates two closely related ground crickets. Evolution **48:** 705–710.

Hawthorne, D. J., 2001 AFLP-based genetic linkage map of the Colorado potato beetle *Leptinotarsa decemlineata*: sex chromo-

somes and a pyrethroid-resistance candidate gene. Genetics **158:** 695–700.

Hawthorne, D. J., and S. Via, 2001 Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature **412:** 904–907.

Hunt, G. J., and R. E. Page, Jr., 1995 Linkage map of the honey bee, *Apis mellifera*, based on RAPD markers. Genetics **139:** 1371–1382.

Jiang, C., and Z-B. Zeng, 1997 Mapping quantitative trait loci with dominant and missing markers in various crosses from two inbred lines. Genetica **101:** 47–58.

Kim, S.-C., and L. H. Rieseberg, 1999 Genetic architecture of species differences in annual sunflowers: implications for adaptive trait introgression. Genetics **153:** 965–977.

Knott, S. A., D. B. Neale, M. M. Sewell and C. S. Haley, 1997 Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. Theor. Appl. Genet. **94:** 810–820.

Kocher, T. D., W. J. Lee, H. Sobolewska, D. Penman and B. McAndrew, 1998 A genetic linkage map of a cichlid fish, the Tilapia (*Oreochromis niloticus*). Genetics **148:** 1225–1232.

Lande, R., 1981 Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. USA **78:** 3721–3725.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly *et al.*, 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

Laurent, V., E. Wajnberg, B. Mangin, T. Schiex, C. Gaspin *et al.*, 1998 A composite genetic map of the parasitoid wasp *Trichogramma brassicae* based on RAPD markers. Genetics **150:** 275–282.

Lu, Z.-X., B. Sosinski, G. L. Reighard, W. V. Baird and A. G. Abbott, 1998 Construction of a genetic linkage map and identification of AFLP markers for resistance to root-knot nematodes in peach rootstocks. Genome **41:** 199–207.

Mackill, D. J., Z. Zhang, E. D. Redona and P. M. Colowit, 1996 Level of polymorphism and genetic mapping of AFLP markers in rice. Genome **39:** 969–977.

Mueller, U. G., and Wolfenbarger. L. L., 1999 AFLP genotyping and fingerprinting. Trends Ecol. Evol. **14:** 389–394.

Naruse, K., S. Fukamachi, H. Mitani, M. Kondo, T. Matsuoka *et al.*, 2000 A detailed linkage map of Medaka, *Oryzias latipes*: comparative genomics and genome evolution. Genetics **154:** 1773–1784.

Otte, D., 1994 *The Crickets of Hawaii: Origin, Systematics and Evolution.* The Orthopterists Society, Academy of Natural Sciences of Philadelphia, Philadelphia.

Parsons, Y. M., and K. L. Shaw, 2001 Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. Mol. Ecol. **10:** 1765–1772.

Petrov, D. A., T. A. Sangster, J. S. Johnston, D. L. Hartl and K. L. Shaw, 2000 Evidence for DNA loss as a determinant of genome size. Science **287:** 1060–1062.

Powell, W., W. T. B. Thomas, E. Baird, P. Lawrence, A. Booth *et al.*, 1997 Analysis of quantitative traits in barley by the use of amplified fragment length polymorphisms. Heredity **79:** 48–59.

Ritchie, M. G., and S. D. F. Phillips, 1998 The genetics of sexual isolation, pp. 291–308 in *Endless Forms: Species and Speciation*, edited by D. J. Howard and S. H. Berlocher. Oxford University Press, New York.

Rundle, H. D., L. Nagel, J. Wenrick Boughman and D. Schluter, 2000 Natural selection and parallel speciation in sympatric sticklebacks. Science **287:** 306–308.

Servedio, M. R., 2000 Reinforcement and the genetics of nonrandom mating. Evolution **54:** 21–29.

Shaw, K. L., 1996a Polygenic inheritance of a behavioral phenotype: interspecific genetics of song in the Hawaiian cricket genus *Laupala*. Evolution **50:** 256–266.

Shaw, K. L., 1996b Sequential radiations and patterns of speciation in the Hawaiian cricket genus *Laupala* inferred from DNA sequences. Evolution **50:** 237–255.

Shaw, K. L., 2000 Interspecific genetics of mate recognition: inheritance of female acoustic preference in Hawaiian crickets. Evolution **54:** 1303–1312.

Shaw, K. L., 2003 Conflict between mitochondrial and nuclear DNA phylogenies of a recent species radiation: what mitochondrial reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. USA (in press).

Shaw, K. L., and Y. M. Parsons, 2002 Divergence of mate recognition behavior and its consequences for genetic architectures of speciation. Am. Nat. **159** (Suppl. S): S61–S75.

Tanksley, S. D., 1993 Mapping polygenes. Annu. Rev. Genet. **27:** 205–233.

True, J. R., J. Liu, L. F. Stam, Z-B. Zeng and C. C. Laurie, 1997 Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana*. Evolution **51:** 816–832.

Via, S., 1999 Reproductive isolation between sympatric races of pea aphids. I. Gene flow restriction and habitat choice. Evolution **53:** 1446–1457.

Via, S., and D. J. Hawthorne, 1998 The genetics of speciation: promises and prospects of quantitative trait locus mapping, pp. 352–364 in *Endless Forms: Species and Speciation*, edited by D. J. Howard and S. H. Berlocher. Oxford University Press, New York.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee *et al.*, 1995 AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23:** 4407–4414.

Wang, Y. H., C. E. Thomas and R. A. Dean, 1997 A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (Aflp) markers. Theor. Appl. Genet. **95:** 791–798.

Wells, M. M., and C. S. Henry, 1998 Songs, reproductive isolation, and speciation in cryptic species of insects, pp. 217–233 in *Endless Forms: Species and Speciation*, edited by D. J. Howard and S. H. Berlocher. Oxford University Press, New York.

Wu, C.-I, and M. F. Palopoli, 1994 Genetics of postmating reproductive isolation in animals. Annu. Rev. Genet. **28:** 283–308.

Xie, C., D. D. G. Gessler and S. Xu, 1998 Combining different line crosses for mapping quantitative trait loci using the identical by descent-based variance component method. Genetics **149:** 1139–1146.

Yasukochi, Y., 1998 A dense genetic map of the silkworm, *Bombyx mori*, covering all chromosomes based on 1018 molecular markers. Genetics **150:** 1513–1525.

Zabeau, M., and P. Vos, 1993 *European Patent Application.* Publication no. EP 0534858.

Communicating editor: G. B. Golding