AFLP-Based Genetic Linkage Map of the Colorado Potato Beetle *Leptinotarsa decemlineata*: Sex Chromosomes and a Pyrethroid-Resistance Candidate Gene

David J. Hawthorne

Department of Entomology, University of Maryland, College Park, Maryland 20742

Manuscript received March 7, 2000
Accepted for publication March 2, 2001

ABSTRACT

A genetic linkage map was constructed from an intraspecific cross of the Colorado potato beetle, *Leptinotarsa decemlineata*. This is an initial step toward mapping the loci that underlie important phenotypes associated with insect adaptation to an agroecosystem. The map was made with 172 AFLP and 10 anonymous codominant markers segregating among 74 backcross (BC_1) individuals. Markers were mapped to 18 linkage groups and a subset of the markers with a mean intermarker distance of 11.1 cM is presented. A pyrethroid-resistance candidate gene, *LdVssc1,* was placed onto the map as well. The sex chromosome was identified by exploiting the XO nature of sex determination in this species using patterns of variation at *LdVssc1* and the codominant markers.

UNDERSTANDING the genetic basis of pest adaptation contributes to our understanding of evolution in changing environments and to the management of pest adaptation in agricultural ecosystems. Many pest insects have undergone rapid evolution during the course of their adaptation to the agricultural ecosystem. The traits that evolve might include general features of pest biology such as colonization ability, rapid generation time, and high fecundity (Hawthorne and Via 1994), or they may be focused on particular attributes of their plant hosts and control measures such as insecticides (Roush and McKenzie 1987; Heinrichs and Rapusas 1990; Heckel *et al.* 1997). As with all adaptive evolution, this process requires selection of phenotypes and a genetic basis to the phenotypic variation under selection. Pest adaptations to a particular agroecosystem provide a unique opportunity to observe both the process and the genetic architecture of selection response under conditions of natural selection.

The Colorado potato beetle [*Leptinotarsa decemlineata* (Say), hereafter CPB] is an important pest of potato (*Solanum tuberosum* L.) in North America, Europe, and Asia. It is native to North America but new to potato, shifting from its ancestral hosts (a suite of *Solanum* spp.) in the mid-19th century (Casagrande 1987; Jacques 1988). The geographical range of CPB has expanded from central and northern Mexico and the southern plains of the United States to now include virtually all potato-growing regions of the world. Populations of this insect have evolved resistance to many synthetic insecticides, including representatives of all major classes of compounds deployed before the 1990s (Argentine *et al.* 1989; Roush *et al.* 1990). Because of its status as a threat to a food staple, much ecological, physiological, and toxicological work has already been done, especially regarding this insect's interactions with environmental compounds (both plant and human produced), pathogens, and parasitoids. Genetic aspects of this insect were first studied by Tower (1906) in what may have been the first (albeit possibly discredited) studies of mutation in animals (Sokoloff 1966). Karyotypic analysis reveals 17 autosomes and an XO sex determination system in which males are XO and females XX (Hsiao and Hsiao 1983).

The linkage map presented here was constructed primarily with amplified fragment length polymorphisms (AFLPs) as the genetic markers. AFLP is a PCR-based method that offers an efficient and reproducible means of generating the genetic markers needed for linkage map construction (Vos *et al.* 1995). AFLP screens many loci quickly and reveals large numbers of polymorphisms. No previous genetic information on the study organism is necessary before use of AFLP and, because it is a PCR-based marker, small amounts of template DNA are sufficient for analysis. These characteristics invite comparison to randomly amplified polymorphic DNAs (RAPDs), another easy-to-use marker (Williams *et al.* 1990). Although a number of excellent linkage maps have been constructed using RAPD polymorphisms (Grattapaglia and Sederoff 1994; Hunt and Page 1995; Bradshaw *et al.* 1998; Beeman and Brown 1999), several researchers have reported difficulty in reproducing RAPD genotypes (Riedy *et al.* 1992; Penner *et al.* 1993). AFLP offers improved reproducibility compared to RAPD because of more stringent reaction

Address for correspondence: Department of Entomology, 4112 Plant Sciences Bldg., University of Maryland, College Park, MD 20742. E-mail: dh176@umail.umd.edu

conditions (Vos *et al.* 1995). Both AFLP and RAPD are dominant markers; among the F_2 offspring from a cross of different homozygotes (*e.g.*, AA and aa) the dominant homozygote (AA) and heterozygote (Aa) are indistinguishable. The reduced information content of dominant markers is not a large liability in mapping studies that utilize a backcross or testcross design; many marker loci will have only two genotypes segregating among the backcross offspring: heterozygotes (Aa) and recessive homozygotes (aa).

Here I report the mapping of AFLP and sequence-tagged AFLP fragments to 18 different linkage groups in CPB. The development of this linkage map lays an important foundation for future genomics research on the CPB, providing a means for localizing genes of ecological importance and revealing the genetic architecture of response to natural selection. The immediate purposes of this work are (1) to estimate recombinational distance between markers for use in quantitative trait loci (QTL) mapping and population genetics, (2) to develop codominant anchor loci for future mapping, (3) to identify the sex chromosome and develop reliable markers on that chromosome, and (4) to place a candidate locus for resistance to pyrethroid insecticides (*LdVssc*) onto the linkage map.

TABLE 1

The sequences of adapters, primers, and primer combinations

The sequences of *Eco*RI and *Pst*I adapters
*Eco*RI adapter
5′-AATTGGTACGCAGTC-3′
5′-CTCGTAGACTGCGTACC-3′
*Pst*I adapter
5′-TGTACGCAGTCTTAC-3′
5′-CTCGTAGACTGCGTACATGCA-3′
The core sequence of primers for *Eco*RI and *Pst*I amplicons
*Eco*RI core: 5′-GACTGCGTACCAATTC-3′
*Pst*I core: 5′-GACTGCGTACATGCAG-3′
Primer overhangs for selective amplification
Primer overhangs added to *Eco*RI core
1 = AA; 2 = AG; 3 = TG; 4 = TC; 5 = CA; 6 = CT;
7 = CG; 8 = CC; 9 = GT; 10 = GC; 11 = GG;
12 = ACT
Primer overhangs added to *Pst*I core
A = ACA ; B = ACC; C = AGG; D = AG;
Primer combinations used
A1 A2 A3 A4 A6 A7 A8 A9 A11
B2 B3 B4 B5 B6 B7 B8 B9 B10 B11
C4 C5 C10
D12

MATERIALS AND METHODS

Beetle sources and genetic crosses: This study describes a backcross analysis of CPB from two pest populations [Long Island, NY (LI) and Freeville, NY (FR)] that differ significantly in their susceptibility to a number of insecticides (Roush *et al.* 1990). Each population originated from a single egg mass of ~30 individuals. Parental beetles, a LI male and a virgin FR female, were mated and their offspring reared to adulthood on greenhouse-grown potato plants in whole-plant sleeve cages. An F_1 female was mated to a second LI male and the backcross offspring (BC_1) were reared to pupation, also on caged potatoes. Late fourth instar larvae were placed into a covered pupation chamber (28 cm × 54 cm × 6.25 cm) containing 6 cm of moist potting soil into which larvae readily burrowed and pupated. Adults were collected as they emerged from the soil and were housed in filter paper-lined petri dishes for 1 day before they were weighed and then sexed (Pelletier 1990). A total of 74 backcross individuals (33 males, 46 females) were genotyped to construct the linkage map.

AFLP construction: AFLP was first described by Vos *et al.* (1995) as a means of amplifying a random array of restriction fragments. The methods of Vos *et al.* (1995) have been modified here. During the early development of AFLP for this insect it became clear that too many bands were produced using the standard six-base recognition restriction enzyme (six-cutter) + four-cutter strategy. To remedy this condition, AFLPs were constructed from fragments created by two six-cutters, *Pst*I and *Eco*RI, both to reduce the fragment number and to bias restriction toward nonmethylated DNA. Frozen insects were homogenized and template DNA was extracted using a commercial protocol (QIAamp tissue kit, QIAGEN, Chatsworth, CA). DNA was eluted into 400 μl of the supplied buffer and stored at −20°. AFLP constructs were assembled in a single-tube reaction by mixing 2.0 μg of genomic DNA, New England Biolabs (Beverly, MA) #4 restriction enzyme buffer [20 mm tris-acetate, 10 mm magnesium acetate, 50 mm potassium acetate, 1 mm dithiothreitol (DTT)], 1.8 mm DTT, 2 mm ATP, 100 ng/μl bovine serum albumen, 20 units *Pst*I, 20 units *Eco*RI, 6 units T4 DNA ligase, and 5 pm of each double-stranded adapter (Table 1). The tubes were incubated for 14 hr at 37°. During this reaction the restriction enzymes create "sticky ends" to which the adapters are ligated. Adapter ligation destroys the restriction site and prevents subsequent cleavage.

Amplification: A two-step amplification strategy was used, similar to that described by Vos *et al.* (1995). The first step (preamplification) is a PCR with core primers that are complementary to the adapter sequence only (Table 1). The second amplification uses longer selective primers, which have two or three overhanging nucleotides at the 3′ end (Table 1). The preamplification round was standard PCR [20 mm Tris-HCl (pH 8.4), 50 mm KCl, 1.5 mm $MgCl_2$, 0.2 mm dNTPs, 1 unit Taq DNA polymerase] that included 2 μl of the AFLP construction as template and 5 pm of each core primer (Table 1). This reaction was cycled 20 times for 1 min at 94°, 1 min at 56°, and 1 min at 72°. Preamplification products were diluted 1:2 with 10 mm Tris (pH 8.0) and stored at −20°. The second step, selective amplification, was also standard PCR (same reagents and concentrations), which included 1 μl of the diluted preamplification product as template, and one each of the *Eco*RI and *Pst*I selective primers (Table 1). The reaction profile was a touchdown-PCR in which the annealing temperature was high (65°) for the first round and then reduced 0.7° for each of the next 12 cycles. The denaturing and extension stages for each cycle were 94° for 10 sec and 72° for 90 sec, respectively. This ramping-down of the annealing temperature was followed by 25 cycles of 94° for 10 sec, 56° for 40 + 1 sec per cycle, and 72° for 90 sec. Following selective amplification, an equal volume of loading dye (98% formamide, 10 mm EDTA, 0.025% xylene cynol, 0.025% bromophenol blue) was added to each sample. After denaturing at 95° for 2.5 min, 1–3 μl were loaded on a 4.3% denaturing polyacrylamide gel (30 × 40 cm). Gels were run for 4 hr at a constant 60 W and then silver stained (Promega, Madison, WI). The amplified fragments were visualized on a light box, recorded as present

or absent, and digitally archived by scanning the gel directly. Two individuals scored all gels, and a third person reviewing archived images resolved discrepancies.

Marker-discovery attributes: To evaluate the usefulness of AFLP for the CPB, the abundance of AFLP polymorphisms was recorded for a random sample of five AFLP reactions performed on the entire backcross family. The numbers of bands per gel, polymorphic bands, and segregating informative bands were recorded.

Genotyping: Linkage analysis was performed on the markers generated from 23 AFLP reactions (Table 1). Markers were included in the analysis if they displayed a segregation pattern consistent with the backcross of a heterozygous F_1 female to a homozygous recessive "tester" male (Aa × aa). In practice, AFLP bands were scored as present or absent in the BC_1s only if they were present in the LI or FR parent, absent in the other, present in the F_1, and absent in the backcross tester parent (LI male). All backcross offspring (BC_1) inherited one allele at each locus from the backcross parent and one from the FR or LI parent via the F_1. Using the "A-H" scoring scheme in Mapmaker 3.0 (Lander *et al.* 1987), BC_1 individuals with markers from the LI parent were scored as "A" and those with markers from the FR parent as "H."

Segregation analysis: Mendelian inheritance of all markers was tested by chi-square, with significance levels corrected for multiple comparisons ($\alpha/173$ markers) to give an experiment-wise $\alpha = 0.05$ ($\chi^2 > 13.1$). MAPMAKER 3.0 was used to infer linkage relationships and to determine the marker order within linkage groups. Linkage was determined at LOD ≥ 3.0 at a maximum (Kosambi) distance of 30 cM and marker order was determined at LOD ≥ 2.0. Only 1 of the 173 markers could not be assigned to a linkage group at LOD ≥ 3.0. A subset of the assigned markers, which were approximately evenly spaced and could be ordered unambiguously at LOD ≥ 2.0 (Figure 1), was selected.

Cloning of AFLP bands: Single-locus markers were developed from AFLP loci at known map positions to act as anchors to a particular linkage group. Bands were isolated directly from the silver-stained gels, reamplified to increase their abundance, and ligated into the 2.9-kb TA cloning vector (pCR vector; Invitrogen, San Diego) according to the manufacturer's protocol (Cho *et al.* 1996). Cloned fragments were sequenced and primers were designed to amplify the cloned fragment from genomic DNA. The resulting amplified fragments were evaluated for size polymorphism (using agarose or denaturing polyacrylamide gel electrophoresis) or sequence polymorphism using double-stranded conformational polymorphism (DSCP; Atkinson and Adams 1997). If markers segregated appropriately as codominant polymorphisms, they were placed on the linkage map using the "try" command in MAPMAKER.

Placement of the candidate gene *LdVssc1*: Using primers published by Lee *et al.* (1999), a 349-bp fragment of the voltage-sensitive sodium channel *LdVssc1* gene, including the functionally significant L1014F mutation site, was amplified from all members of the mapping family. This locus is similar to the Drosophila sodium channel gene *para*, which has been shown to contribute to target-site insensitivity to pyrethroid insecticides in CPB and other pest insects (Dong and Scott 1994; Guerrero *et al.* 1997; Park *et al.* 1997). Fragments were evaluated by DSCP and genotypes of all individuals were determined.

RESULTS

AFLP marker-discovery attributes: The six + six-cutter strategy reduced the number of bands per gel sufficiently to resolve individual bands using +2 and +3 base overhanging primers. The five reactions evaluated for marker attributes had an average of 72.4 bands on each gel. Fifty percent of the bands were polymorphic within the backcross family, and 14.4% of the bands were segregating appropriately for use in the linkage analysis. Band sizes ranged from ~200 to 1500 bp, with most in the 300 to 900-bp range. As expected, fragments are larger than those previously reported for another insect, the fall armyworm (McMichael and Prowell 1999), in which the six-cutter + four-cutter strategy was used. The 23 AFLP reactions generated 173 informative markers. The expected segregation ratio of each marker in the BC_1 generation is 1:1 and none differed significantly from this ratio (all $\chi^2 < 13.1$).

Linkage map construction: MAPMAKER inferred 18 linkage groups from the backcross data (Figure 1). Because the haploid chromosome number of CPB is 18 (Hsiao and Hsiao 1983), our linkage groups appear to correspond to chromosomes. Of the 173 AFLP markers used to make the map, positions of 86 well-spaced AFLPs, 10 codominant markers derived from AFLPs, and the *LdVssc1* fragment are presented here. The map covers 1032 cM with a mean (±SD) intermarker distance of 11.1 cM (±6.72).

Cloned AFLP fragments: I chose 10 AFLP markers that segregated in the backcross population and occurred on different linkage groups in preliminary analyses to be cloned. Table 2 lists the primer sequences and fragment sizes for the cloned markers. Map locations of these single-locus PCR markers are indicated on Figure 1. All single-locus markers were found on the same linkage groups as their progenitor AFLP marker (Figure 1), with differences in recombination distance between loci most likely due to genotyping error in the AFLP analysis. Sequences of single-locus markers have been submitted to GenBank (accession nos. AYO28388–AYO28397).

Placement of candidate gene *LdVssc1*: All individuals in the mapping population were genotyped using the fragment of *LdVssc1* described by Lee *et al.* (1999). The "try" command in MAPMAKER was used to place this fragment on linkage group 11 (LOD = 8.21), and its map-order position on linkage group 11 (Figure 1) was supported by a LOD score of 4.11.

Determination of the X chromosome: The CPB, like many insects, has an XO system of sex determination in which females are diploid (XX) and males are haploid (XO). This systematic difference in ploidy was exploited to distinguish the X chromosome from the autosomes, which are all diploid for males and females. Previous work (Argentine *et al.* 1989; Heim *et al.* 1992) suggested that pyrethroid resistance is sex linked, offering a starting point for our search (as the candidate gene for pyrethroid resistance, *LdVssc1*, mapped to linkage group 11). The genotypes at *LdVssc1* of the F_1 and BC parent were Aa and aa, respectively. If this locus is on the X chromosome there will be four genotypes of BC_1 offspring in which the males are A_ or a_ and females Aa

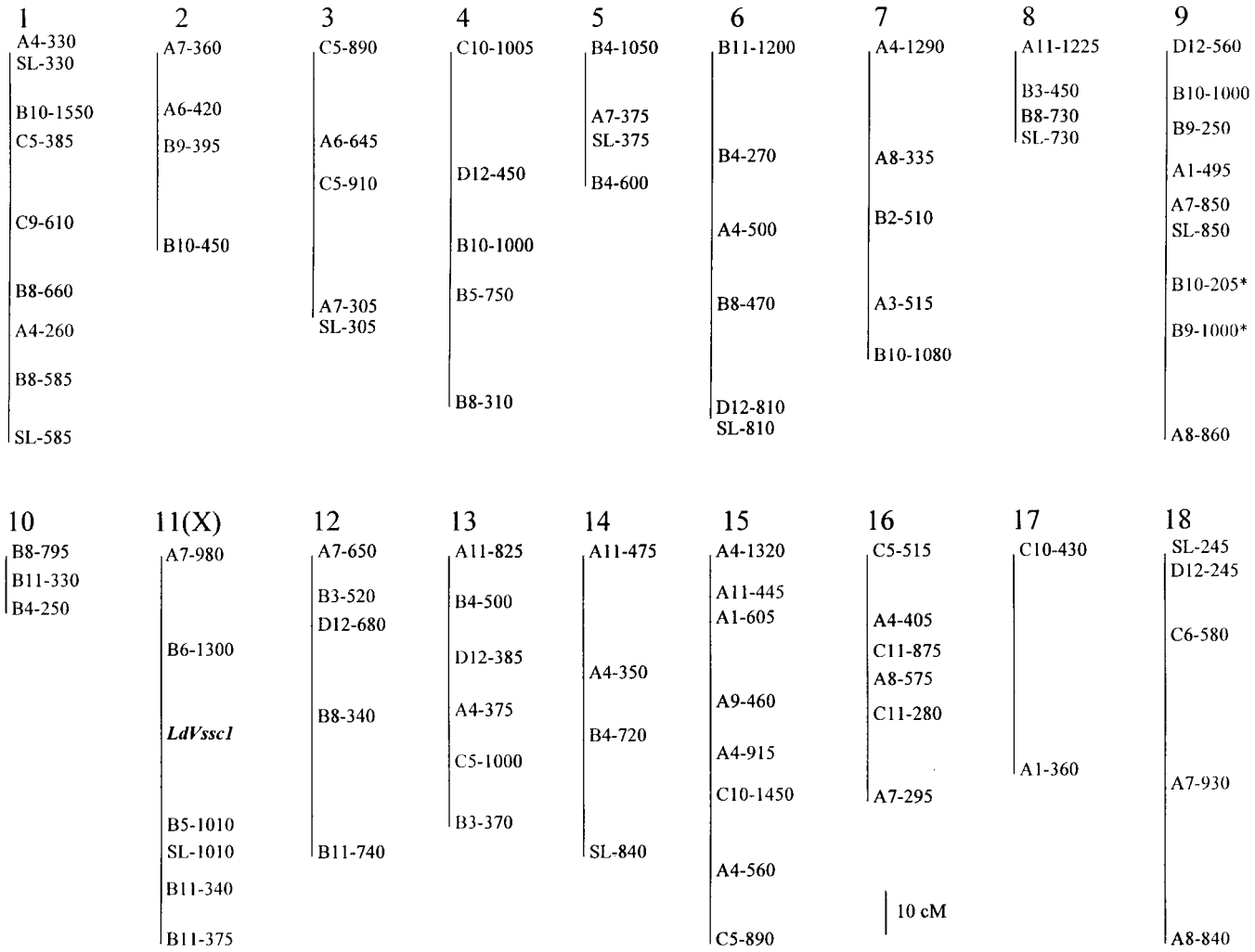

FIGURE 1.—Linkage map of the CPB genome. AFLP marker loci are labeled as described in Table 1; single-locus markers are named with the approximate size of the progenitor AFLP marker and prefaced by "SL." Two markers indicated with an asterisk are ordered at LOD ≥ 1.6; all others are ordered at LOD ≥ 2.0.

or aa. Using DSCP to visualize the amplified fragments, a single band may be due to homozygosity or hemizygosity. Therefore, the "aa" and the "a" genotypes are indistinguishable and cannot be used to distinguish XX individuals from XO. However, all "Aa" individuals (21/21) were female and all "A" individuals (17/17) were male, fitting the pattern expected if *LdVssc1* were on the X chromosome. A second marker, the codominant SL-1010, also maps to linkage group 11. Using DSCP again, 43 of 44 (98%) BC_1 females were heterozygous (diploid), while 29 of 30 (97%) males were hemizygous, with all hemizygous males receiving their allele from the maternal and none from the paternal parent. None of the other nine single-locus codominant markers mapping to different linkage groups displayed sex-related differences in banding pattern. As an additional check, 20 field-collected beetles were sexed and genotyped at the SL-1010 locus. Whereas 9 of 10 females were demonstrably diploid (two bands), all 10 males had a single band, suggesting haploidy. These results strongly indicate that linkage group 11 corresponds to the X chromosome.

DISCUSSION

By considering the evolution of ecologically important traits in pest insects much can be learned about the genetic basis of phenotypic evolution, especially for populations faced with strong selection. Remarkably, the genetic basis of adaptation in natural populations is largely unknown (ORR 1998). The use of genetic linkage maps can help identify the loci contributing to adaptive changes in populations. Maps with a density of markers around 20–30 cM are sufficient for detecting the presence of QTL with increased marker density allowing more precise positioning of the QTL (DARVASI and SOLLER 1994; LYNCH and WALSH 1998). The 11-cM map presented here will allow efficient QTL mapping in this insect. One important use of genetic linkage maps is comparison of positions of mapped candidate loci

TABLE 2

Names, primer sequences, and characteristics of single-locus markers

Marker name	Primer direction	Primer Sequences (5′–3′)	Fragment size (bp)	Annealing temperature	[MgCl] (mM)
SL_1010	Forward	GCTTTCATACAACTCTACAGTTG	956	47	1.5
	Reverse	CATCAAAGACCTTCTGCGGAG			
SL_810	Forward	GACAAATGGATTACACTTACATGG	657	47	1.3
	Reverse	GCGTGTGAGCCATTATCAGCAG			
SL_375	Forward	TGGTTTCAGATTTGTCCCAG	310	45	1.5
	Reverse	GGTCCCACCCACTAGATG			
SL_850	Forward	CACCAAAGTCGCTTGTGAGTGGA	701	51	1.5
	Reverse	GCTCAGTAAAAACCTTCTTCAGGG			
SL_305	Forward	GTTTATGAAATCAAATATCATTCC	260	45	1.5
	Reverse	GTAGATCCTTATTGTAAAGAGAC			
SL_840	Forward	CCTTATACTGGCATGTTGCACG	308	48	1.5
	Reverse	CCATTCTCGATGAAAGAGTCAG			
SL_245	Forward	TGAGGCTGAAGTCGTCCGTAC	219	49	2.0
	Reverse	GGACGTCGCAAATTTGACCAC			
SL_330	Forward	GAGCCAGAAAATCCCTGGC	283	45	2
	Reverse	GATTTCTCAAAAGGCTCTGTAG			
SL_585	Forward	CTCGACATAGGCGTCGGCA	531	49	2.0
	Reverse	CTCATTAGCCACTCGTCAGTG			
SL_730	Forward	ACGTACCTTGAACGTAAGTG	658	43	1.5
	Reverse	GCGAAATAGTTCTTCACGAC			

with QTL. Such comparison will streamline efforts to associate ecologically important genetic variation with known genes. This study confirms that a candidate gene, *LdVssc1*, previously shown to contribute a large degree of resistance to pyrethroid insecticides (Lee *et al.* 1999), is located on the X chromosome (linkage group 11) in the CPB. The resistant phenotype will therefore be expressed in all males carrying the resistance-conferring allele, even if it is recessive, providing a boost to the rate of increase of a favored allele especially when that allele is rare. Now, we can ask whether QTL associated with resistance to pyrethroid insecticides are found at or near this locus on linkage group 11 and whether genetically correlated resistances to other insecticides are due to pleiotropic effects of that locus.

The CPB linkage map will also contribute to population genetic goals. For example, knowing the recombinational distance between a locus that has undergone strong selection (such as *LdVssc1*) and other markers allows us to measure the effect of that selection on nucleotide variation at linked loci (*e.g.*, Yan *et al.* 1998). This will further efforts to develop relationships between genetic diversity, strength of selection, and genetic distance from the locus under selection in contemporary populations.

AFLP seems especially well suited for linkage map construction using intraspecific crosses. In this study, variation was so abundant that marker screening was unnecessary. The mean number of AFLP markers identified per gel (7.4) was much higher than that observed using RAPDs on *Apis melifera* (Hunt and Page 1995; 2.8 bands per gel) or on *Tribolium castaneum* (Beeman and Brown 1999; 1.5 bands per gel). However, because CPB populations may have inherently higher levels of genetic polymorphism than either Apis or Tribolium, these differences may not be due solely to the markers.

Use of AFLP for mapping represents a fundamentally different approach to identifying and mapping loci than that typically used with RFLP or microsatellites. Construction of linkage maps with AFLP allows us to discover and localize markers of interest and then invest in converting them into codominant markers. In contrast, RFLP or microsatellite approaches require us to invest in marker development first and then determine if they are useful. Following this strategy here, I have cloned and sequenced several AFLP fragments, converting them to single-locus codominant markers. These features make AFLP potentially useful in many types of population studies and may increase the accessibility of genetic information for genetically unstudied species.

Andrea Badgley, Maria Loosen, Stasia Skillman, and Mike Jackson helped in the lab, and Dale Moyer and Ward Tingey supplied insects. Fred Gould, George Kennedy, and Susan McCouch provided lots of early inspiration. Jeffrey Feder, John McKenzie, Yvonne Parsons, Don Price, and Kerry Shaw made many helpful comments. I especially thank Richard Harrison who generously provided research space and guidance during the initial phases of this work. This work was first supported by the Center for Integrated Pest Management and then by grant no. 58-1275-6-034 from the U.S. Department of Agriculture.

LITERATURE CITED

Argentine, J. A., J. M. Clark and D. N. Ferro, 1989 Genetics and synergism of resistance to azinphosmethyl and permethrin in the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. **82:** 698–705.

Atkinson, L., and E. A. Adams, 1997 Double-strand conformation polymorphism (DSCP) analysis of the mitochondrial control region generates highly variable markers for population studies in a social insect. Insect Mol. Biol. **6:** 369–376.

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

Bradshaw, Jr., H. D., K. G. Otto, B. E. Frewen, J. K. McKay and D. W. Schemske, 1998 Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (Mimulus). Genetics **149:** 367–382.

Casagrande, R. A., 1987 The Colorado potato beetle: 125 years of mismanagement. Bull. Entomol. Soc. Amer. **33:** 142–150.

Cho, Y. G., M. W. Blair, O. Panaud and S. R. McCouch, 1996 Cloning and mapping of variety specific rice genomic DNA sequences: amplified fragment-length polymorphism (AFLP) from silver stained polyacrylamide gels. Genome **39:** 373–378.

Darvasi, A., and M. Soller, 1994 Optimum spacing of genetic markers for determining linkage between marker loci and quantitative trait loci. Theor. Appl. Genet. **89:** 351–357.

Dong, K., and J. G. Scott, 1994 Linkage of *kdr*-type resistance and the *para*-homologous sodium channel gene in German cockroaches (*Blatella germanica*). Insect Biochem. Mol. Biol. **24:** 647–654.

Grattapaglia, D., and R. Sederoff, 1994 Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics **137:** 1121–1137.

Guerrero, F. D., F. D. Jamroz, D. Kammlah and S. E. Kunz, 1997 Toxicological and molecular characterization of pyrethroid-resistant horn flies, *Haematobia irritans*: identification of *kdr* and *super-kdr* point mutations. Insect Biochem. Mol. Biol. **27:** 745–755.

Hawthorne, D. J., and S. Via, 1994 Variation in performance on two grape cultivars within and among populations of grape phylloxera from wild and cultivated grapes. Entomol. Exp. Appl. **70:** 63–76.

Heckel, D. G., L. C. Gahan, F. Gould and A. Anderson, 1997 Identification of a linkage group with a major effect on resistance to Bacillus thuringiensis Cry1Ac endotoxin in the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. **90:** 75–86.

Heim, D. C., G. G. Kennedy, F. L. Gould and J. W. van Duyn, 1992 Inheritance of fenvalerate and carbofuran resistance in Colorado potato beetles—*Leptinotarsa decemlineata* (Say)—from North Carolina. Pestic. Sci. **34:** 303–311.

Heinrichs, E. A., and P. Rapusas, 1990 Response to selection for virulence of Nephotettix virescens (Homoptera cicadellidae) on resistant rice cultivars. Environ. Entomol. **19:** 167–175.

Hsiao, T. H., and C. Hsiao, 1983 Chromosomal analysis of *Leptinotarsa* and *Labidomera* species (Coleoptera: Chrysomelidae). Genetica **60:** 139–150.

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

Jacques, R. L., Jr., 1988 *The Potato Beetles: the Genus Leptinotarsa in North America (Coleoptera: Chrysomelidae)*. E. J. Brill, New York.

Lander, E. S., P. Green, J. Abrahamson, A. Blarlow, 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.

Lee, S. H., J. B. Dunn, J. M. Clark and D. M. Soderland, 1999 Molecular analysis of *kdr*-like resistance in a permethrin-resistant strain of Colorado potato beetle. Pestic. Biochem. Physiol. **63:** 63–75.

Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

McMichael, M., and D. P. Prowell, 1999 Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Ann. Entomol. Soc. Am. **92:** 175–181.

Orr, H. A., 1998 The population genetics of adaptation: the distribution of factors fixed during adaptative evolution. Evolution **52:** 935–948.

Park, Y., M. F. J. Taylor and R. Feyereisen, 1997 A valine421 to methionine mutation in IS6 of *hscp* voltage-gated sodium channel associated with pyrethroid resistance in *Heliothis virescens* F. Biochem. Biophys. Res. Commun. **239:** 688–691.

Pelletier, Y., 1990 Sexing of the Colorado potato beetle, Leptinotarsa decemlineata (Say), in Proceedings Acadian Entomological Society, 50th Annual Meeting, Fredericton, New Brunswick, Canada, p. 38.

Penner, G. A., A. Bush, R. Wise, W. Kim, L. Domier *et al.*, 1993 Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods App. **2:** 341–345.

Riedy, M. F., W. J. Hamilton, III and C. F. Aquadro, 1992 Excess of non-parental bands in offspring from known primate pedigree assayed using RAPD PCR. Nucleic Acids Res. **20:** 918.

Roush, R. T., and J. McKenzie, 1987 Ecological genetics of insecticide and acaricide resistance. Annu. Rev. Entomol. **32:** 361–380.

Roush, R. T., C. W. Hoy, D. W. Ferro and W. M. Tingey, 1990 Insecticide resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae): influence of crop rotation and insecticide use. J. Econ. Entomol. **83:** 315–319.

Sokoloff, A., 1966 *The Genetics of Tribolium and Related Species*. Academic Press, New York.

Tower, W. L., 1906 *An Investigation of Evolution in Chrysomelid Beetles of the Genus Leptinotarsa*. Carnegie Institute, Washington, DC. Publ. **48:** 1–320.

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.

Williams, J. G., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey, 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:** 6531–6535.

Yan, G., D. Chadee and D. W. Severson, 1998 Molecular population genetics of the yellow fever mosquito: evidence for genetic hitchhiking effects associated with insecticide resistance. Genetics **148:** 793–800.

Communicating editor: G. A. Churchill