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Table 2. Recombination fractions and standard errors for RFLP marker data

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<tr>
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<th>LF188a</th>
<th>LF217</th>
<th>D6.12</th>
<th>LF178</th>
<th>Ace</th>
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<td>LF217</td>
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Table 3. Recombination fractions and standard errors for phenotypic marker data

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Standard errors in parentheses.

*a JoinMap-derived composite data from Hitchen and Wood (1975b) and Lockhart et al. (1970).
*b Two-point comparisons not available.

(french-Constant et al. 1992). The three main insecticide targets are (1) acetylcholinesterase or AChE, the target for organophosphorus (OP) and carbamate insecticides; (2) the γ-aminobutyric acid (GABA) receptor gene Resistance to dieldrin or Rdl, the site of action of cyclodiene insecticides; and (3) the para sodium channel gene, the target for DDT and pyrethroids. In all three of these genes, single- or multiple-point mutations appear to cause target site insensitivity. A number of point mutations in the Drosophila Ace gene have been shown to affect the sensitivity of AChE inhibition by OP and carbamate insecticides (Mutero et al. 1994). In contrast, a single amino acid replacement in the GABA receptor gene Rdl causes resistance to cyclodiene in a wide range of insects (french-Constant et al. 1993; Thompson et al. 1993b). A single amino acid replacement in para gene homologues also appears to cause knockdown resistance (kdr) to DDT (Reenan R, Ganetzky BS, Pitendrigh BR, and french-Constant RH, unpublished data). The role of these target sites in genes for A. aegypti resistance has however not been investigated.

In order to investigate the importance of target site insensitivity in A. aegypti we were interested in attempting to correlate the recombinational map position of previously identified resistance loci with molecular probes for these three target sites. In this study molecular probes for these three genes were used as RFLP markers to determine their genome positions and subsequently to correlate the location of the insecticide target genes with the resistance phenotypes previously mapped by simple recombinational mapping. Here we report that the map positions of both the Rdl and para gene homologues correlate with the previously described locations for the cyclodiene and DDT/pyrethroid resistance loci, respectively. Interestingly, although insensitivity to AChE has not been reported for A. aegypti, the Ace gene maps as a single locus in close proximity to the sex-determining locus. We discuss the implications of these results for the relative importance of target site insensitivity in mosquito insecticide resistance.

Table 4. Segregation of the Ace locus relative to sex determination in the F1 from a cross between Hamburg and Moyo-ln-Dry strains

<table>
<thead>
<tr>
<th></th>
<th>No. of Individuals</th>
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<tr>
<td>Sex</td>
<td>P1</td>
</tr>
<tr>
<td>Female</td>
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</tr>
<tr>
<td>Male</td>
<td>0</td>
</tr>
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</table>

*Phenotypic designation: P1 = Hamburg male type; H = heterozygote; P2 = Moyo-ln-Dry male type.
Materials and Methods

Cloning of Insecticide Resistance Genes

The cloning of the Rdl homologue from A. aegypti via the low stringency screening of an adult cDNA library has been previously reported (Thompson et al. 1993a). The Ace gene homologue was cloned from A. aegypti cDNA via the use of degenerate primers in the polymerase chain reaction (Anthony et al. 1995). The 89 bp para gene homologue probe was cloned from genomic DNA using degenerate PCR primers in the procedure of Knipple et al. (1991).

Mosquito Crosses, DNA Isolation, and Southern Blotting

RFLP genetic data for the Ace and para loci are based on F₂ intercross progeny from a pairwise mating between the A. aegypti Hamburg (HAM) and Moyo-In-Dry (MOY) strains. The origins of these strains and RFLP-based estimates of genetic diversity between them are described elsewhere (Severson et al. 1994b). Mosquitoes were reared as previously described (Christensen and Sutherland 1984). DNA isolation from individual mosquitoes, digestion with EcoRI, Southern blotting, and hybridizations were performed as previously described (Severson et al. 1993, 1994a).

Linkage Analysis

Chi-square goodness-of-fit values for the HAM × MOY F₂ intercross population were calculated for segregation and independent assortment ratios for all pairs of loci. Multipoint linkage analyses were performed using the JoinMap computer program (Stam 1993). A minimum LOD threshold of 3 was used to identify linkage between markers. Recombination frequencies were converted into map distances (cM) using the Kosambi (1944) function. Composite linkage maps also were constructed using the JoinMap program. A composite RFLP map was prepared from all available RFLP mapping data; for reference purposes we included data for RFLP markers that define the ends of each linkage group. The Rdl locus composite map included previously published RFLP genetic data involving this locus (Severson et al. 1993, 1994a). The composite DDT and DI loci maps included phenotypic marker data extracted from the literature (Hitchen and Wood 1974, 1975a,b; Lockhart et al. 1970; Malcolm and Wood 1982). Because cross-resistance to DDT and pyrethroids is provided by a single locus on chromosome 3 (Malcolm 1983), we combined the independently reported mapping data for this locus.

Results

Segregation Ratios and Recombination Fractions

Segregation ratios for the HAM × MOY F₂ population are shown in Table 1. The RFLP loci generally fit the expected 1:2:1 segregation ratios, with the exception that a slight excess of heterozygotes was obtained for the LF396 locus on chromosome 3. Recombination frequencies and standard errors for the three insecticide resistance genes and RFLP markers are shown in Table 2. JoinMap-derived recombination fractions and standard errors for insecticide resistance phenotypes and some mutant marker phenotypes are shown in Table 3.

Linkage Analysis

The map position of the Ace locus in the HAM × MOY F₂ population and the marker positions relative to the composite RFLP linkage map for chromosome 2 are shown in Figure 1. No evidence for recombination between the Ace locus and sex determi-
Rdl gene is responsible for cyclodiene resistance in Aedes aegypti. Also included are the map positions of the Rdl resistance phenotype on chromosome 2. This suggests that the loci map to the same chromosome region.

Figure 2. Map distances are indicated in Kosambi centiMorgans.

Figure 3. RFLP genetic linkage map for the para locus in a Hamburg × Moyo-Dry F2 intercross population, a phenotypic linkage map for the DDT locus based on recombination fractions extracted from the literature (Hitchen and Wood 1975a, Malcolm and Wood 1982), and marker positions relative to the composite RFLP linkage map for chromosome 3 in A. aegypti. Map distances are indicated in Kosambi centiMorgans.

Discussion

Cyclodiene resistance is inherited as a single gene with a semidominant resistance phenotype in A. aegypti (Lockhart et al. 1970). RFLP mapping of the Rdl cDNA shows a good correlation with the localization of the resistance locus DI on chromosome 2. This result is consistent with our previous observation that cyclodiene-resistant A. aegypti carry the same alanine to serine amino acid replacement in the Rdl GABA receptor gene as other cyclodiene/picrotoxin-resistant insects (Thompson et al. 1993a). Further, A. aegypti Rdl GABA receptors mutated to the resistance associated serine show insensitivity to picrotoxin when expressed in baculovirus-infected insect cells (Shotkoski et al. 1994). There is therefore considerable evidence that the originally mapped cyclodiene locus DI is indeed the Rdl GABA receptor gene.

The para sodium channel gene was originally cloned from D. melanogaster (Loughney et al. 1989). Linkage between para gene homologues and knock down resistance (kdr) to pyrethroids has been shown in both houseflies (Kipple et al. 1994; Williamson et al. 1993) and cockroaches (Dong and Scott 1994). Resistance is associated with a single-point mutation in kdr-type alleles in houseflies (Williamson et al. 1996) and cockroaches (Miyazaki et al. 1996), and with an additional point mutation in super-kdr houseflies (Williamson et al. 1996). The A. aegypti para gene homologue probe we used maps to chromosome 3, in the same location as a locus conferring resistance to DDT and pyrethroids. That this locus confers cross-resistance to both DDT and pyrethroids (Malcolm 1983) is especially interesting because this cross-resistance spectrum is also characteristic of kdr resistance in houseflies. We can therefore infer that the DDT/pyrethroid locus on chromosome 3 is associated with target site insensitivity and that an additional locus associated with DDT resistance on chromosome 2 in A. aegypti is probably associated with increased metabolic activity, probably mediated by glutathione-S-transferase or cytochrome P450 activity.

Finally, although the Ace gene homologue did not map to any known resistance locus, it did map very close to the sex-determining locus. Insensitivity to AChE has been reported in a number of different mosquito species (french-Constat and Bonning 1989), but not in A. aegypti. To date however, no sex-linked insensitive AChE has been reported for any mosquito species. There are two hypotheses to explain these observations: (1) there are two Ace loci in A. aegypti, as

The map position of the para locus in the HAM × MOY F2 population and the marker positions relative to the composite RFLP linkage map for chromosome 3 are shown in Figure 3. Also included is the map position of the DDT with cross-resistance to pyrethroids loci (DDTpy) relative to two mutant marker loci, black-tarsus (blt) and miniature-body (min). Comparative map positions indicate that the para gene and the DDT resistance locus map to the same general chromosome region on chromosome 3. This suggests that DDT resistance in A. aegypti is determined by the para gene.

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Discussion

Cyclodiene resistance is inherited as a single gene with a semidominant resistance
suggested for anopheline mosquitoes (Banks et al. 1996), but only the autosomal copy of Ace is potentially associated with resistance, or (2) A. aegypti only carries a single Ace locus, and if detected, resistance associated with insensitivity to AChE will be sex linked, in contrast with other mosquito species. At present our data support the second hypothesis, because examinations of Southern blots probed with the A. aegypti Ace gene only reveals evidence for a single sex-linked Ace locus in this species.

This study suggests that of the resistance traits mapped in A. aegypti, two of the most widespread resistance mechanisms, cyclodiene and DDT/pyrethroid resistance, are associated with target site insensitivity. Knowledge of the mechanisms of insecticide resistance is useful to the formulation of control strategies. Thus insensitivity of the para target site to DDT will confer cross-resistance to pyrethroids. The spraying of DDT may, therefore, have reduced the potential efficacy of pyrethroids even before they had been introduced. Finally, identification of the point mutations conferring target site insensitivity, which therefore delimit insecticide binding sites themselves, may aid in the rational design of new insecticides to overcome existing resistance mechanisms.

From the Departments of Animal Health and Biomedical Sciences (Severson) and Entomology (Anthony, Anderd, and Frech-Constant), University of Wisconsin, Madison, Wisconsin. We thank V. Kassner and Y. Zhang for technical assistance. G. Yan for critical comments on the manuscript, and M. Radite for artwork. This work was supported by grants AI28781 (D.W.S. and R.Rf-C.), AI33127 (D.W.S.) AI53437 (D.W.S.), and AI55026 (R.Rf-C.) from the National Institutes of Health. Please address reprint requests to Dr. David W. Severson, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.

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References


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Comparative Mapping of Anchor Loci from HSA19 to Cattle Chromosomes 7 and 18

Q. Gao and E. J. Womack

Six loci—CALR, EPOR, JUNB, JUND, CEA, and PRKCG—were assigned to bovine chromosomes using PCR-based hybrid somatic cell analysis. The five genes other than CALR are comparative mapping anchor loci. This study, together with the previous assignment of three anchor loci—INSR, LDLR, APOE—and four other genes—AMH, GPI, RYR1, LHB—defines the conserved syntenic relationship between human chromosome 19 and cattle chromosomes 7 and 18. Genes on HSA19p13.3-13.2 are conserved in cattle chromosome 7, while those on HSA19- q13.1-13.4 are conserved in cattle chromosome 18. In contrast, homologous genes from HSA19 are located on four different mouse chromosomes, namely MMU10, MMU8, MMU9, and MMU7. This is further evidence that syntenic conservation between cattle and human generally exceeds that observed between hu- man and mouse.

The rapid development of gene maps in various species has made comparative gene mapping a valuable tool for the study of genome organization and chromosome evolution. Comparative genome analysis is primarily based on the mapping of in- dividual conserved loci in different spe-