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Comparative linkage maps for the mosquitoes, *Aedes albopictus* and *Ae. aegypti*, based on common RFLP loci

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Abstract

***Aedes albopictus* and *Aedes aegypti* are members of the mosquito family Culicidae and share a haploid chromosome complement of three. Although a genetic linkage map based on restriction fragment length polymorphism (RFLP), markers exists for *Ae. aegypti*, the extent of synteny and linkage order conservation between the two species was unknown. A comparative linkage map for *Ae. albopictus* was constructed based mainly on cDNA clones from *Ae. aegypti*. Nearly all *Ae. aegypti* probes hybridized to *Ae. albopictus* DNA at high stringency. For eighteen RFLP markers tested, the linkage group and linear order appears to be identical for the two species. 78% of the loci tested exhibited significant deviations from the expected segregation ratio in at least one of the test crosses. An excess of heterozygote genotypes was recovered with most loci. This probably reflects the effects of lethal loci on survival of F_2 progeny homozygous for the parental genotypes. These results demonstrate that comparative linkage maps based on common DNA markers provide a basis for rapidly developing linkage maps for various mosquito species, and the opportunity to examine the significance and function of orthologous quantitative trait loci associated with mosquito vector competence for disease transmission.**

Keywords: genetic linkage, synteny, chromosome evolution, genetic similarity.

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Introduction

The mosquito family, Culicidae, is divided into three subfamilies Anophelinae, Culicinae and Toxorhynchitinae, that comprise about 3100 recognized species. The haploid complement of three chromosomes is conserved across virtually all mosquito species. However, genome size varies from about 0.23 to 1.90 pg (see Kumar & Rai, 1993). This variability is largely due to differences in levels of repetitive DNA (Black & Rai, 1988).

Genetic linkage information, based on mutant markers or isozyme markers, is available for only a few mosquito species. Although the disciplines of genetics for these species remain largely independent, there is evidence of synteny both within and across subfamilies (Green, 1982; Hunt, 1987; Kumar & Rai, 1990a; Matthews & Munstermann, 1990, 1994). Identification of conserved syntenies could facilitate integration of genetic information among species, and provide a mechanism for rapidly developing linkage maps for genetically uncharacterized species. In-depth comparative linkage studies in mosquitoes have been limited because few isozyme or mutant marker loci segregate in a given cross.

The development of DNA sequences as genetic markers provides the technology to develop detailed linkage maps for mosquitoes and to evaluate genome organization of orthologous loci across species (see Severson, 1994). Comparative linkage maps, based on common restriction fragment length polymorphism (RFLP) markers, have been developed for maize with rice (Ahn & Tanksley, 1993), sugarcane (D'Hont *et al.*, 1994), and sorghum (Pereira *et al.*, 1994), for tomato with potato (Bonierbale *et al.*, 1988) and pepper (Tanksley *et al.*, 1988), for garden pea with lentil (Weeden *et al.*, 1992) and for human with mouse (Nadeau *et al.*, 1992).

The mosquitoes *Aedes albopictus* and *Aedes aegypti* originated in East Asia and sub-Saharan Africa, respectively (Smith, 1956; Mattingly, 1957). Both species are in the *Stegomyia* subgenus, but belong to different groups within the subgenus; *Ae. albopictus* is a member of the Albopictus Subgroup of the Scutellaris Group, whereas *Ae. aegypti* is a member of the Aegypti Group (see Rai *et al.*, 1982). Crossing experiments clearly demonstrated incompatibility between the two species (Leahy & Craig, 1967). We

previously described a RFLP linkage map for the mosquito *Ae. aegypti* (Severson *et al.*, 1993) and herein we report the construction of comparative linkage maps for *Ae. albopictus* and *Ae. aegypti* based on a common set of RFLP markers.

Results and Discussion

We previously demonstrated that sixteen randomly selected *Ae. aegypti* probes hybridized with *Ae. albopictus* genomic DNA under high stringency conditions (Severson *et al.*, 1994a). In the present study we screened an additional forty clones; *in toto* we have observed hybridization with fifty-five of fifty-six clones tested, indicative of a high level of gene conservation between the two species. Additionally, ribosomal DNA clones (Kumar & Rai, 1990a) and some middle repetitive DNA clones (Kumar & Rai, 1991a, b, 1992), isolated from *Ae. albopictus*, have been successfully hybridized with genomic DNA of *Ae. aegypti* and several other mosquito species.

We observed relatively high levels of genetic diversity between the *Ae. albopictus* strains using *Ae. aegypti* clones. For example, twenty-seven of fifty-five clones are informative for RFLP linkage mapping (Table 1). That is, the two strains share no common restriction fragments when examined with these clones. For the remainder of the clones, the two strains share one or more common restriction fragments. With our laboratory's most genetically diverse *Ae. aegypti* strains, Moyo-In-Dry and Gambia (Severson *et al.*, 1994a), nineteen of these same clones were informative (Table 1). We also previously reported very low levels of genetic similarity between several *Culex pipiens* strains based on *Ae. aegypti* markers (Severson *et al.*, 1994a).

For eighteen clones, F_2 progeny were scored with respect to the parental RFLP as homozygous for the NO or SAK genotypes or as heterozygotes. Autoradiograms representing three clones which exhibit typical hybridization patterns are shown in Fig. 1. Significant deviations from the expected 1:2:1 ratio were observed with fourteen (77.8%) of the loci examined in at least one of the test crosses (Table 2). With most loci we recovered an abundance of the heterozygote genotype. We reported a similar phenomenon with *Ae. aegypti* (Severson *et al.*, 1994c). This probably reflects the effects of lethal loci on survival of F_2 progeny homozygous for the parental genotypes; balanced lethal systems apparently promote and maintain heterozygosity in mosquito strains (Matthews & Craig, 1989; Munstermann, 1994).

By hybridizing DNA from segregating populations of *Ae. albopictus* with *Ae. aegypti* clones it was possible to construct a comparative linkage map (Fig. 2). The *Ae. aegypti* linkage map shown represents the best fit, using the JoinMap computer program (Stam, 1993), for all available RFLP mapping data (Severson *et al.*, 1993, 1994b, c, and unpublished data). All eighteen RFLP markers examined with *Ae. albopictus* mapped to the same linkage group and retained the same linear order as that observed with *Ae. aegypti*.

The loci mapped in *Ae. albopictus* cover 98.4 cM, whereas the same loci cover 129.1 cM in *Ae. aegypti* (Fig. 2). The greatest variability was observed in recombination frequencies of loci common to chromosome 1 in both species. The map distance between LF90 and TY7 was 18.2 cM versus 43.7 cM for *Ae. albopictus* and *Ae. aegypti*, respectively. We observed similar low recombination frequencies for chromosome 1 loci for *Ae. albopictus* in some preliminary studies (unpublished data). Since the nuclear DNA content of *Ae. albopictus* is equal to or greater than that of *Ae. aegypti* (Rao & Rai, 1987) and their chromosome 1 morphologies are similar (Motara & Rai, 1978), the basis for the differences in recombination frequencies is unclear. A possible influence is the variability in nuclear DNA content among *Ae. albopictus* populations; Kumar & Rai (1990b) reported nearly 3-fold variability in haploid nuclear DNA content among thirty-seven populations. Crosses between individuals with large differences in heterochromatin content could reflect an influence on meiotic pairing of homologous chromosomes and, consequently, recombination frequencies.

Although some localized rearrangements may be identified subsequently, these results clearly demonstrate that chromosomal positions for other RFLP loci can be directly predicted for *Ae. albopictus*, based on the *Ae. aegypti* linkage map. Therefore, if the general concept of whole arm rearrangement for chromosome evolution in mosquitoes is correct (see Matthews & Munstermann, 1994), it should be possible to rapidly determine the general genome organization in most mosquito species with fifteen to twenty markers and thereafter predict loci positions. This

Table 1. Utility of *Aedes aegypti* probes for comparative mapping with *Aedes albopictus*[*]

Species	Strains	Informative loci	Mixed allele loci	Informative (%)
Aedes albopictus	New Orleans × Sakamoto	27	28	49.1
Aedes aegypti	Moyo-In-Dry × Gambia	19	36	34.5

[*]Informative loci, no restriction fragments shared; mixed allele loci, one or more restriction fragments shared.

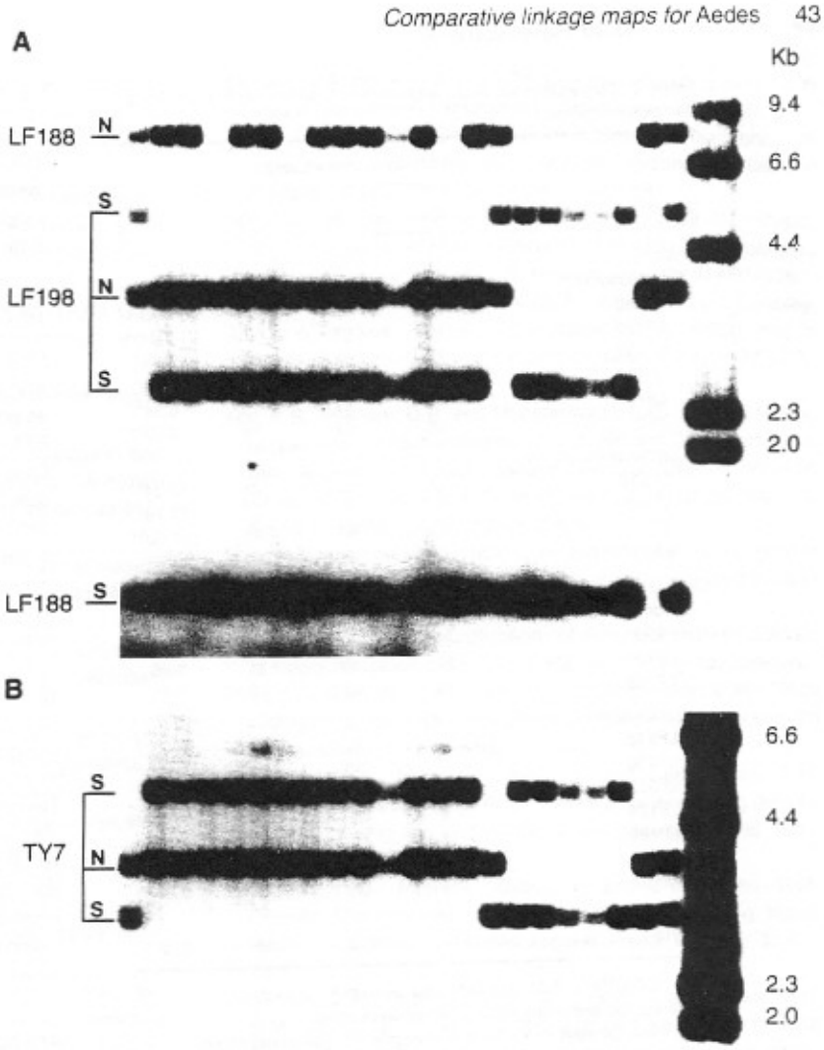

Figure 1. RFLP observed in twenty-two *Aedes albopictus* F_2 progeny following hybridization with *Aedes aegypti* probes. (A) segregation patterns following simultaneous hybridization with clones LF188 and LF198; (B) segregation patterns when the same progeny were hybridized with clone TY7. RFLP alleles representing the parental New Orleans strain (N) and Sakamoto (S) strain are indicated. The three loci are all located on chromosome 1. The two right-hand lanes = *Hind*III-digested lambda phage DNA markers.

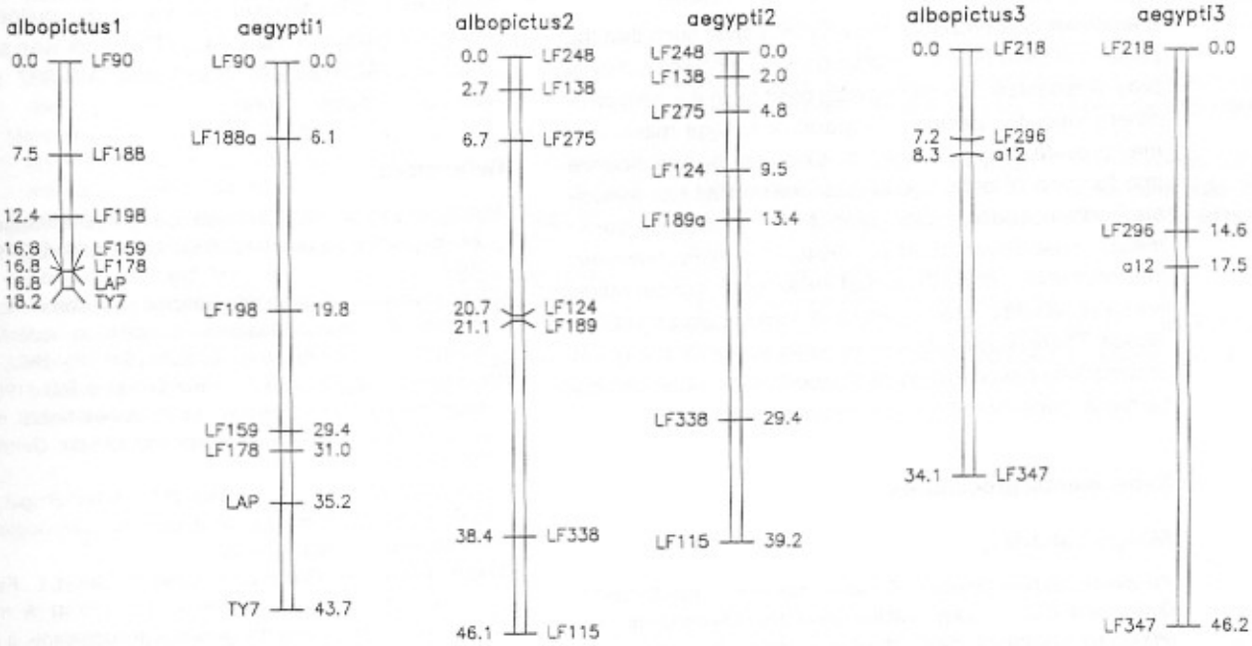

Figure 2. Comparative genetic maps of *Aedes albopictus* and *Aedes aegypti* based on common RFLP loci. Map distances are listed in Kosambi centiMorgans.

Table 2. Segregation of *Aedes aegypti* RFLP markers in F_2 from *Aedes albopictus* crosses.†

	No. of individuals‡			
Marker	P_1	H	P_2	χ^2
Cross 1				
Chromosome 1				
LF188	20	60	20	4.00
LF198	19	65	15	9.94**
LF159	18	61	19	6.34*
LF178	18	61	19	6.34*
LAP	18	61	19	6.34*
TY7	18	64	16	9.27**
Chromosome 3				
LF218	10	62	28	12.24**
LF296	9	61	27	12.70**
a12	10	60	28	11.83**
LF347	13	43	30	6.59*
Cross 2				
Chromosome 1				
LF90	20	39	13	1.86
LF188	21	40	11	3.66
LF198	20	42	9	5.91
Chromosome 2				
LF248	5	41	30	16.93**
LF138	7	39	30	13.98**
LF275	9	37	30	11.66**
LF124	9	56	11	17.16**
LF189	10	55	11	15.24**
LF338	16	45	15	2.60
LF115	19	39	17	0.24
Chromosome 3				
a12	14	44	18	2.32

*$P < 0.05$; **$P < 0.01$ (loci tested for expected 1:2:1 ratio).
†Marker loci are arranged in chromosomal order.
‡P_1, New Orleans strain; H, heterozygote; P_2, Sakamoto strain.

phenomenon is probably mutually inclusive, such that the position of DNA markers mapped in *Ae. albopictus* should provide a basis for predicting map position in *Ae. aegypti* or other mosquito species. Comparative linkage maps also may provide the opportunity to examine the significance and function of orthologous quantitative trait loci associated with mosquito vector competence for disease transmission (see Severson *et al.*, 1994c). It is likely, however, that high levels of synteny and linear order conservation will be observed primarily with DNA markers utilizing cDNA clones. There is no evidence presently available to suggest that markers based on nontranscribed DNA sequences will be highly conserved between mosquito species.

Experimental procedures

Mosquito strains

Ae. albopictus New Orleans (NO) strain was kindly provided by Dr G. Scoles and Dr G. Craig, Jr at the University of Notre Dame. *Ae. albopictus* Sakamoto (SAK) strain was obtained by one of us (A.M.) while at Nagasaki University. Because *Ae. albopictus* was apparently recently introduced into the US from Japan (Hawley *et al.*, 1987; Kambhampati *et al.*, 1991), both the NO and SAK strains represent populations originating from Japan. The number of colonized generations is unknown for these strains. Mosquitoes were reared using standard procedures (Christensen & Sutherland, 1984). Genetic data are based on (NO × SAK)F_2 progeny from pairwise matings.

RFLP and linkage analysis

DNA extraction from individual and bulked mosquitoes, digestion with *Eco*RI, Southern blotting and hybridizations were performed as previously described (Severson *et al.*, 1993, 1994a). All of the RFLP markers used in this study are *Ae. aegypti* cDNA or genomic DNA clones. Individual clones were initially hybridized to 10 μg bulk DNA digests of both *Ae. albopictus* strains to verify hybridization under high-stringency conditions and to identify informative clones for linkage mapping. Eighteen informative RFLP markers were selected for hybridization with (NO × SAK)F_2 progeny. These markers cover the *Ae. aegypti* genome at 10–20 cM intervals (Severson *et al.*, 1993, 1994b, c, and unpublished data).

Chi-square goodness-of-fit values were calculated for segregation and independent assortment ratios for all pairs of loci. Multipoint linkage analyses were performed using the MapMaker computer program (Lander *et al.*, 1987). A minimum LOD threshold of 3 was used to identify linkage between markers. Recombination frequencies were converted into map distances (cM) using the Kosambi function (Kosambi, 1994). A composite RFLP linkage map was developed for chromosome 1, based on the recombination frequencies observed with two independent crosses, using the JoinMap computer program (Stam, 1993). Comparative linkage maps for *Ae. albopictus* and *Ae. aegypti* were drawn using the DrawMap computer program (van Ooijen, 1994).

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