Restriction Fragment Length Polymorphism Mapping of Quantitative Trait Loci for Malaria Parasite Susceptibility in the Mosquito *Aedes aegypti*

D. W. Severson, V. Thathy, A. Mori,[1] Y. Zhang and B. M. Christensen

Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, Wisconsin 53706

Manuscript received May 27, 1994
Accepted for publication December 17, 1994

ABSTRACT

Susceptibility of the mosquito *Aedes aegypti* to the malarial parasite *Plasmodium gallinaceum* was investigated as a quantitative trait using restriction fragment length polymorphisms (RFLP). Two F_2 populations of mosquitoes were independently prepared from pairwise matings between a highly susceptible and a refractory strain of *A. aegypti*. RFLP were tested for association with oocyst development on the mosquito midgut. Two putative quantitative trait loci (QTL) were identified that significantly affect susceptibility. One QTL, *pgs*[*2,LF98*], is located on chromosome *2* and accounted for 65 and 49% of the observed phenotypic variance in the two populations, respectively. A second QTL, *pgs*[*3,MalI*], is located on chromosome *3* and accounted for 14 and 10% of the observed phenotypic variance in the two populations, respectively. Both QTL exhibit a partial dominance effect on susceptibility, wherein the dominance effect is derived from the refractory parent. No indication of epistasis between these QTL was detected. Evidence suggests that either a tightly linked cluster of independent genes or a single locus affecting susceptibility to various mosquito-borne parasites and pathogens has evolved near the *LF98* locus; in addition to *P. gallinaceum* susceptibility, this general genome region has previously been implicated in susceptibility to the filarial nematode *Brugia malayi* and the yellow fever virus.

MALARIA maintenance and transmission among warm-blooded vertebrate animals are totally dependent upon the availability of competent mosquito intermediate hosts. Malarial parasites, Plasmodium spp., infect the mosquito after ingestion in a bloodmeal obtained from an infected vertebrate. Plasmodium must complete critical aspects of their life cycle, including gametogenesis and eventual development to vertebrate infective sporozoites, within the complex physiological mileu presented by the mosquito (WARBURG and MILLER 1991). Gamete maturation and fertilization to produce motile ookinetes occur within the mosquito midgut after gametocyte acquisition by female mosquitoes during blood-feeding. The ookinetes must migrate through the peritrophic matrix, penetrate the midgut epithelium and subsequently form oocysts. Oocysts undergo sporogony to produce sporozoites that must migrate to and invade the mosquito salivary glands. However, individual mosquito species, as well as individual mosquitoes within species, vary significantly in their competency to support development of the various Plasmodium spp. (BOYD 1949).

The conflicting literature regarding Plasmodium susceptibility in various mosquito species suggests a complex mode of inheritance. HUFF (1929, 1931) reported that susceptibility of *Culex pipiens* to *P. cathemerium* was heritable as an apparently recessive trait, yet only 65% of the individuals were susceptible after three generations of selection. Conversely, DENNHÖFER (1971) reported that *C. pipiens* susceptibility to *P. cathemerium* was due to a single gene with incomplete dominance. With *Anopheles stephensi*, refractoriness to *P. gallinaceum* was associated with a single, dominant locus (FRIZZI *et al.* 1975). With *A. gambiae*, although susceptibility to *P. berghei* was correlated with an incompletely dominant character, the influence of interacting polygenes was suggested (AL-MASHHADANI *et al.* 1980). GRAVES and CURTIS (1982) reported that refractoriness of *A. gambiae* to *P. yoelii nigeriensis* showed incomplete dominance, but their low susceptibility *A. gambiae* strains were highly susceptible to *P. falciparum*. WARD (1963) suggested that *A. aegypti* susceptibility to *P. gallinaceum* was primarily determined by a single locus with incomplete dominance but was unable to fix a strain for refractoriness despite 26 generations of selection. KILAMA and CRAIG (1969) subsequently reported that *A. aegypti* susceptibility to *P. gallinaceum* was determined by a dominant gene, *pls*, located on chromosome *2*; however, to justify monofactorial inheritance they needed to invoke partial penetrance in the refractory phenotype. That is, individuals were considered refractory if they allowed development of 10 or less oocysts. We recently reported development of two *A. aegypti* strains that are highly refractory to and of intermediate susceptibility to *P. gallinaceum*, respectively (THATHY *et al.* 1994). Crosses involving these strains and highly susceptible strains suggested that susceptibility is largely determined by a

Corresponding author: David W. Severson, Department of Animal Health and Biomedical Sciences, 1655 Linden Dr., University of Wisconsin, Madison, WI 53706.

[1] *Present address:* Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia.

major gene exhibiting at least partial dominance; however, these results also suggested that this locus either exhibits allelic variability or that additional modifier genes are involved.

Although *A. aegypti* is not a vector for human Plasmodium spp., it is genetically the best characterized mosquito species and is the primary natural vector for the avian malarial parasite, *P. gallinaceum*. Since the primary human malarial parasite, *P. falciparum*, shares a closer phylogenetic relationship with *P. gallinaceum* than with other human, murine or primate malarias (WATERS *et al.* 1991), elucidation of the genetic basis for vector competency in the *A. aegypti*/*P. gallinceum* system could provide significant insight concerning the genetic relationship between *P. falciparum* and its Anopheles spp. vectors. Our success in constructing a genetic linkage map for *A. aegypti*, based on restriction fragment length polymorphism (RFLP) markers, provides the tools to partition genetic aspects of malaria parasite susceptibility into discrete Mendelian components (SEVERSON *et al.* 1993). In the present study, quantitative trait loci (QTL) were sought for the genetic factors determining *P. gallinaceum* susceptibility (pgs) in *A. aegypti* using RFLP markers. We have identified a QTL consistent with the *pls* locus on chromosome *2* and an additional QTL affecting pgs on chromosome *3*.

MATERIALS AND METHODS

Mosquito crosses and parasites: Genetic data are based on F_2 intercross progeny from pairwise matings between the *P. gallinaceum*-susceptible *A. aegypti* RED strain and a *P. gallinaceum*-refractory *A. aegypti* strain selected from the Moyo-In-Dry strain (MOYO-R). Selection of the MOYO-R strain and relative susceptibilities of the MOYO-R and RED strains are described by THATHY *et al.* (1994). Two independently generated populations (T1 and T2) involving (RED × MOYO-R) F_2 progeny were prepared using RED as the female. The origins of the RED and MOYO-IN-DRY strains and RFLP-based estimates of genetic diversity between them are described elsewhere (SEVERSON *et al.* 1994a). Both strains are type form, *A. aegypti aegypti*. The RED strain carried the red-eye (*re*) locus on chromosome *1*, the spot-abdomen (*s*) locus on chromosome *2* and the black-tarsus (*blt*) locus on chromosome *3*. Mosquitoes were reared as previously described (CHRISTENSEN and SUTHERLAND 1984).

F_2 female mosquitoes were allowed to engorge on restrained White Leghorn chicks that were naturally infected with *P. gallinaceum* essentially as described by KILAMA and CRAIG (1969) with some modifications (THATHY *et al.* 1994). Engorged females were dissected 6–7 days after blood-feeding to determine whether they were permissive for *P. gallinaceum* development. The number of oocytes that had successfully developed on the midgut of individual mosquitoes was counted.

RFLP and statistical analysis: DNA extraction from individual mosquitoes, digestion with *Eco*RI, Southern blotting and hybridizations were performed as previously described (SEVERSON *et al.* 1993, 1994a). Sixteen informative RFLP probes were selected to provide maximum coverage of the three *A. aegypti* chromosomes at 10–20-cM intervals, based on known chromosomal position (SEVERSON *et al.* 1993, 1994b and SEVERSON, D. W., unpublished data).

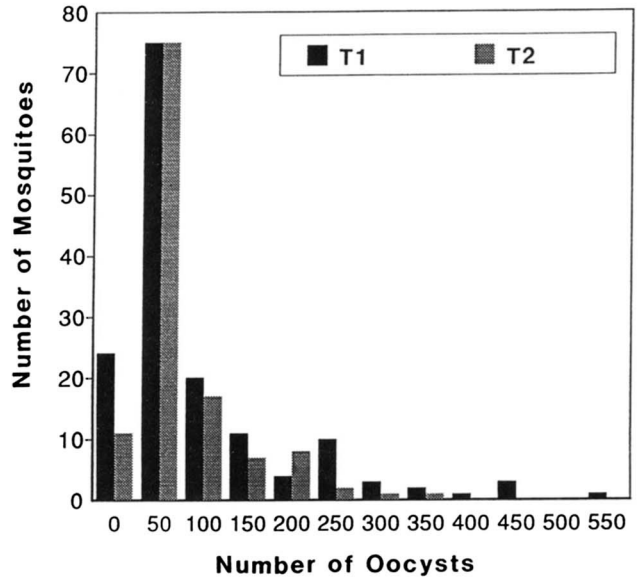

FIGURE 1.—Distribution of phenotypes for pgs in two F_2 populations of female *A. aegypti* mosquitoes.

A composite RFLP genetic linkage map was developed based on the recombination frequencies between markers determined from this and previous studies (SEVERSON *et al.* 1993, 1994b and SEVERSON, D. W., unpublished data). This map was assembled using the JoinMap computer program (STAM 1993) and reflects the best fit for all available RFLP mapping data. Recombination frequencies were converted into map distances (cM) using the Kosambi function (KOSAMBI 1944).

Multipoint linkage analysis and QTL mapping were conducted using the MAPMAKER/QTL computer package (LANDER *et al.* 1987; LINCOLN and LANDER 1990). Cube-root transformations of oocyst counts were done to normalize the data. A LOD score of 2.0 was used as the threshold for declaring the presence of a QTL. Individual QTL positions were identified following a previously described format (SEVERSON *et al.* 1994b): pgs[*n*,*y*], where pgs = *P. gallinaceum* susceptibility, n = the chromosome number and y = the RFLP marker with the greatest independent R^2 value. The effects of putative QTL were assessed using linear regression procedures (SAS Institute 1990); to examine the type of gene action, polynomial models were fit using the three genotypic classes at the RFLP locus exhibiting the greatest R^2 value at each QTL. In this context, a good fit with a linear component is interpreted as evidence for additive gene action. A good fit with a quadratic component, which accounts for curvature in the data, is interpreted as evidence for dominance effects.

RESULTS

Phenotypic variability in Plasmodium susceptibility: The oocyst frequency distribution was determined for two populations of *P. gallinaceum*-challenged F_2 female mosquitoes (Figure 1). These populations represented two independently prepared crosses between the *P. gallinaceum*-susceptible RED strain and a *P. gallinaceum* refractory MOYO-R strain. Mean oocyst counts and within population SDs were 67.9 ± 103.4 (n = 154) and 45.7 ± 64.5 (n = 122) for populations T1 and T2, respectively.

TABLE 1

Segregation of markers in the F_2 from two populations evaluated for Plasmodium susceptibility

		Population			
		T1		T2	
Chromosome	Marker	Segregation ratio	χ^2	Segregation ratio	χ^2
1	*LF235*	48:86:20	12.4**	55:52:15	28.2**
	LF198	47:87:20	12.2**	43:64:15	13.1**
	LF178	45:98:11	26.8**	39:79:4	30.9**
2	*LF248*	29:85:40	3.4	24:81:17	14.5**
	LF138	29:83:38	3.0	25:80:17	13.4**
	s[a]	30:124	2.6	25:97	1.4
	LF272	32:93:28	6.9*	23:79:20	11.3**
	LF282	33:92:28	6.2*	23:75:24	6.4*
	LF98	37:83:33	1.2	20:77:24	8.7*
	VCP	36:85:29	3.6	22:79:21	11.2**
	LF250	36:88:29	3.8	18:80:24	12.6**
3	*b1t*[a]	38:116	0.0	13:107	12.8**
	LF218	37:89:24	7.8*	12:80:30	17.6**
	LF261	41:87:26	5.7	6:84:32	28.9**
	LF108	41:79:25	4.5	4:88:30	35.5**
	LF168	47:79:27	5.4	3:95:24	45.8**
	MalI	40:73:26	3.2	6:82:34	27.7**
	LF347	43:75:33	1.3	17:68:37	27.2**

Marker loci are arranged in chromosomal order.
[a] Loci were scored for 1:3 ratio, all others 1:2:1.
* $P < 0.05$, ** $P < 0.01$.

Segregation of RFLP loci: Segregation ratios for each RFLP locus are shown in Table 1. Significant deviations from the expected 1:2:1 for F_2 progeny are evident for all loci on chromosome *1*. With T1, RFLP loci on chromosomes *2* and *3* generally fitted their expected 1:2:1 segregation ratios, with only a slight bias toward the heterozygote genotype. With T2, all loci on chromosomes *2* and *3* exhibited significant deviations from the expected 1:2:1 segregation ratios. An abundance of heterozygotes was observed for all loci on chromosome *2*. All loci on chromosome *3* reflected a deficiency in the RED genotype. This deficiency increased progressively from the linkage group ends to the *LF168* locus, suggesting the presence of a RED-strain-derived genetic lethal within the region of *LF168*.

RFLP analysis of Plasmodium susceptibility: QTL analyses for both populations identify associations between pgs and two independent QTL. QTL plots of LOD scores for chromosomes *2* and *3* provide a basis for estimating the individual QTL positions (Figure 2). Both populations identified a QTL at the interval defined by *LF282* and *LF98* on chromosome *2*. This QTL, designated *pgs*[*2,LF98*], explained the largest fraction (single-locus model) of the observed phenotypic variance in each population (65 and 49% for T1 and T2, respectively). While both populations identified a QTL on chromosome *3*, the interval placement varied. This QTL was defined by adjacent intervals, *LF168* and *MalI vs. MalI* and *LF347*, for T1 and T2, respectively. The phenotypic variance (single-locus model) explained by this QTL, designated *pgs*[*3,MalI*], varied from 14 to 10% for T1 and T2, respectively.

Comparison of QTL genotype with pgs phenotype clearly illustrates their interrelationships (Table 2). A single individual accounts for the apparent increase in mean oocyst numbers observed for the S/R QTL genotype with T1. This individual exhibited the largest oocyst count ($n = 520$) observed during these studies. Because this individual also reflected a recombination event between *MalI* and *LF347*, it seems likely that it was actually heterozygous at the locus affecting susceptibility.

Because of the extensive segregation distortion observed in the T2 population (Table 1), some QTL genotype classes were represented by only a small number of individuals, including in some classes one or zero (Table 2). Therefore, the genetic effects of the individual QTL were assessed with T1 only.

Polynomial regression statistics indicating the significant effects of QTL associated with *LF98* and *LF347* on Plasmodium susceptibility in T1 are shown in Table 3. The linear and quadratic components both significantly reduce the residual variance with both RFLP loci. This suggests that each QTL exhibits a partial dominance effect on Plasmodium susceptibility. Further, this analysis indicates for both QTL that the dominance effect is derived from the refractory parent. Finally, the crossproduct term was not significant ($P > 0.05$), indi-

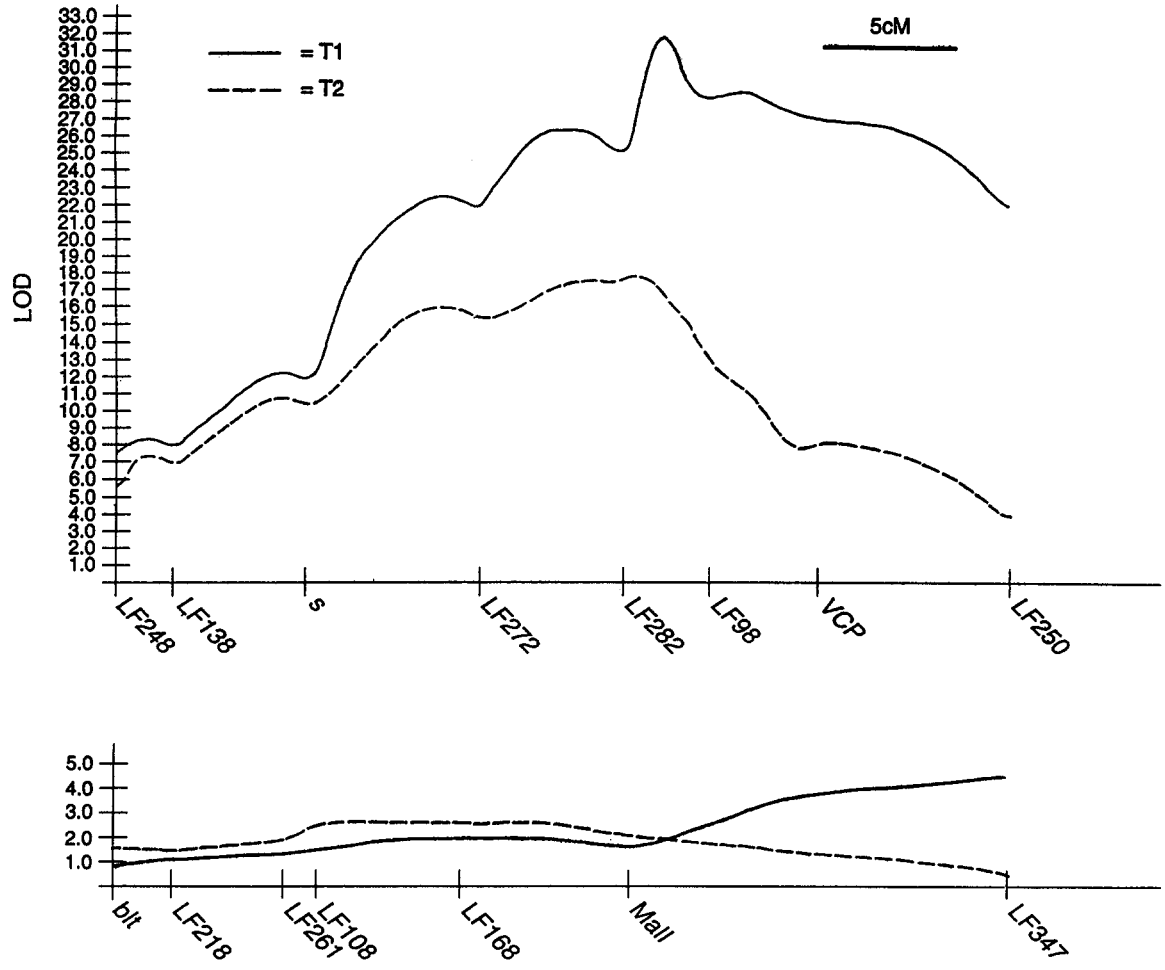

FIGURE 2.—LOD scores indicating QTL likelihood maps for pgs with chromosomes *2* (top) and *3* (bottom) in *A. aegypti* mosquitoes.

cating a lack of epistatic interaction between the two QTL.

DISCUSSION

Our results identify two independent QTL affecting pgs in *A. aegypti*. A major QTL on chromosome *2*, *pgs*[*2,LF98*], accounts for the greatest fraction of the observed variance in pgs. With *pgs*[*2,LF98*], refractoriness exhibits partial dominance over susceptibility. The effect and map location of *pgs*[*2,LF98*] are consistent with the previously described *pls* locus (KILAMA and CRAIG 1969; THATHY *et al.* 1994), even though susceptibility at *pls* was described as being dominant to refractoriness. Since *pls* was evaluated as a qualitative character in backcross progeny in these studies, the reported effect must be viewed with caution. The plus/minus scoring of a complex phenotype in a backcross is inadequate for determining mode of action. In this context, mode of action is best examined by using DNA-based markers to quantitatively evaluate F_2 intercross progeny (see SEVERSON 1994).

The QTL on chromosome *3*, *pgs*[*3,MalI*], while of lesser effect than *pgs*[*2,LF98*], does clearly have a significant yet apparently independent influence on pgs in *A. aegypti*. Also, with *pgs*[*3,MalI*], partial dominance effects are associated with refractoriness. The *A. aegypti* strain selected for an intermediate level of susceptibility to *P. gallinaceum* (MOYO IS) by THATHY *et al.* (1994) was likely fixed for refractoriness at *pgs*[*2,LF98*] and therefore reflects only the effect of *pgs*[*3,MalI*]. In the present study, individuals homozygous for markers linked with refractoriness at *pgs*[*2,LF98*] and with susceptibility at *pgs*[*3,MalI*] carried a mean of 10.3 ± 15.7 oocysts. The MOYO IS line described by THATHY *et al.* (1994) carried a mean of 8.1 ± 9.2 oocysts.

Our results reflect some limits on the precision of estimating the location of individual QTL. For a QTL with a large effect, such as *pgs*[*2,LF98*], we identified a QTL corresponding to the same interval in two independent populations. However, for a QTL with lesser effects such as *pgs*[*3,MalI*], we were able to identify the QTL in both populations, but its location varied from the *MalI-LF347* interval for T1 to the *LF168-MalI* interval for T2. This phenomenon is not surprising given our relatively small sample sizes and the lower

TABLE 2

Association of QTL genotype with pgs in (RED × MOYO-R)F_2 female mosquitoes

QTL genotype		Population			
		T1		T2	
		pgs phenotype			
QTL1[a]	QTL2	*n*	No. of oocysts	*n*	No. of oocysts
S[b]	S	14	261.1 ± 109.8	1	320
S	H	12	134.8 ± 110.7	17	127.4 ± 80.1
S	R	9	187.7 ± 142.2	5	91.6 ± 41.2
H	S	23	69.7 ± 63.9	5	60.8 ± 77.1
H	H	41	28.8 ± 48.9	48	40.9 ± 46.3
H	R	16	15.0 ± 16.7	22	13.6 ± 15.1
R	S	6	10.3 ± 15.7	0	—
R	H	21	1.9 ± 2.9	17	3.2 ± 3.3
R	R	6	2.8 ± 4.7	7	0.6 ± 1.0

RED, *Plasmodium gallinaceum* susceptible; MOYO-R, *Plasmodium gallinaceum* refractory.

[a] QTL1, *pgs* [*2,LF98*], T1 data for *LF98* locus and T2 data for *LF282* locus; QTL2, *pgs* [*3,Mall*], T1 data for *LF347* locus and T2 data for *Mall* loucs.

[b] S, RFLP marker homozygous for the susceptible parental genotype; H, marker heterozygous; R, marker homozygous for the refractory parental genotype.

phenotypic variance (10–14%) attributable to this QTL. While a number of factors influence the accuracy of QTL identification and placement, population size and the magnitude of the genetic effect play highly significant roles in anticipated predictive power. Simulation studies suggest that our population sizes are barely sufficient for detecting QTL that explain 10% or less of the total phenotypic variance (VAN OOIJEN 1992).

The observed distortions in segregation ratios reflect at least two distinct phenomena. First, sex determination in *A. aegypti* is determined by a single autosomal gene on chromosome *1*, with maleness being the dominant allele (GILCHRIST and HALDANE 1947). Therefore, the observed segregation ratios for female F_2 progeny will reflect a bias toward the maternal RFLP genotype with all chromosome *1* loci (SEVERSON *et al.* 1993). Second, although the segregation distortion observed for chromosomes *2* and *3* could simply be due to sampling bias associated with relatively small populations, it likely reflects the effects of lethal loci on survival of F_2 progeny homozygous for one or both parental types. Lethal genes have been reported to promote and maintain heterozygosity in mosquito strains (MATTHEWS and CRAIG 1989; MUNSTERMANN 1994). Given the concordance in statistical analyses between T1 (chromosomes *2* and *3* with only slight evidence for segregation distortion) and T2 (chromosomes *2* and *3* with highly distorted segregation ratios), it is likely that the distortion observed did not influence interpretation of the relationships between QTL and RFLP loci, although it may account for the observed variability in interval placement for *pgs*[*3,Mall*].

TABLE 3

Regression statistics for RFLP markers defining QTL for Plasmodium susceptibility in the T1 population (R^2 = 0.65)

Variable	d.f.	Regression coefficient	SE	*t* value	*P*
Intercept	1	6.05	0.25	23.80	0.0001
LF98					
Linear	1	−3.13	0.44	−7.10	0.0001
Quadratic	1	0.46	0.21	2.21	0.0286
LF347					
Linear	1	−1.60	0.43	−3.69	0.0003
Quadratic	1	0.56	0.21	2.62	0.0098

Evaluated as the effect of adding alleles (0, 1 or 2) from the refractory parent at each locus.

The results of this study may provide some potential insights relative to the evolution of mosquito vector competence. We recently reported that, while susceptibility of *A. aegypti* to the filarial worm parasite *Brugia malayi* was primarily determined by a QTL on chromosome *1*, *fsb*[*1,LF178*], the significant effect of a second QTL located near *LF98* on chromosome *2*, *fsb*[*2,LF98*], also was identified (SEVERSON *et al.* 1994b). Additionally, significant correlations between yellow fever virus susceptibility and gene frequencies among mosquito populations at the *Pgm* isozyme locus have been reported (TABACHNICK *et al.* 1985). The *Pgm* locus resides about four recombination units from the *pls* locus (MUNSTERMANN 1990); this suggests that a locus for yellow fever susceptibility also is linked with the *LF98* locus. Therefore, independent studies involving several *A. aegypti* strains and their susceptibility to a protozoan and a metazoan parasite and an arbovirus all implicated the same genome region on chromosome *2*. We conclude that either a tightly linked cluster of independent

genes or a single locus affecting susceptibility to various mosquito-borne parasites and pathogens has evolved in the genome region near the *LF98* locus.

Plasmodium spp., lymphatic filarioids (Brugia spp. and *Wuchereria bancrofti*) and arboviruses must successfully cross the mosquito midgut to complete development. Midgut barriers to transmission or antagonistic hemolymph factors have been implicated for each of these disease agents (see MITCHELL 1983; CHRISTENSEN 1986; WARBURG and MILLER 1991). This apparent commonality in immune mechanisms could represent the phenotypic expression of the locus or loci linked with *LF98*. This hypothesis is strengthened by a QTL analysis of *A. aegypti* susceptibility to the filarial worm *Dirofilaria immitis;* in two populations, we have identified a major QTL for *D. immitis* susceptibility on chromosome *1* but have found no evidence for any QTL association with *LF98* (D. W. SEVERSON, MORI, A. and CHRISTENSEN, B. W., unpublished data). This parasite migrates down the mosquito gut and into the lumen of the Malphigian tubules where it becomes intracellular and completes development; therefore, *D. immitis* does not traverse the midgut wall and is not exposed to hemolymph until it migrates to the probosis after development to the infective third-stage larva.

These studies need to be expanded with consideration for variance in pgs expression across different genetic backgrounds. That is, because pgs is a quantitative trait, it is probable that allelic variants exist for the two QTL identified in this study and that additional QTL remain to be identified. For example, the effect of *fsb*[*2,LF98*] on *B. malayi* susceptibility is variable depending on the population under consideration (SEVERSON *et al.* 1994b). KILAMA (1973) examined *pls* expression among several geographic strains of *A. aegypti* and reported variability only among African strains. However, for this study susceptibility was evaluated as a single-locus, qualitative character. Our results demonstrate the necessity for evaluation of susceptibility as a quantitative trait.

Of further interest would be to evaluate the role of orthologous loci for the QTL identified for *P. gallinaceum* and *A. aegypti* with other mosquito and Plasmodium species. While some inconsistencies exist, our studies and the literature generally suggest that inheritance of Plasmodium susceptibility is determined primarily by a major gene, with additional effects attributable to undefined modifier genes (see Introduction). Therefore, if the general concept of whole arm homeology for chromosome evolution in mosquitoes is correct (MATTHEWS and MUNSTERMANN 1994), it should be possible to rapidly evaluate the potential for homeologies involving QTL linked with RFLP markers for *A. aegypti*. We provided some support for this hypothesis by demonstrating high levels of homology between our RFLP markers for *A. aegypti* and genomic DNA representing several mosquito species (SEVERSON *et al.* 1994a), and by the development of a comparative linkage map for *A. albopictus* using *A. aegypti* markers (SEVERSON *et al.* 1994c). Comparative linkage mapping has been used successfully to identify homeologies for orthologous RFLP loci and QTL for seed weight in cowpea and mung bean, despite extensive genome rearrangements (FATOKUN *et al.* 1992).

Finally, the identification of RFLP loci linked with genes affecting Plasmodium susceptibility provides the physical landmarks to begin efforts to isolate these genes; map-based cloning is dependent on a series of very logical and independent steps, beginning with the mapping of target genes with DNA markers (WING *et al.* 1994).

We thank J. ROMERO-SEVERSON for assistance with data analyses and critical comments on the manuscript and M. RADTKE for artwork. This work was supported by grant AI-33127 from the National Institutes of Health to D.W.S.

LITERATURE CITED

AL-MASHHADANI, H. M., G. DAVIDSON and C. F. CURTIS, 1980 A genetic study of the susceptibility of *Anopheles gambiae* to *Plasmodium berghei*. Trans. R. Soc. Trop. Med. Hyg. **74:** 585–594.

BOYD, M. F., 1949 *Malariology: A Comprehensive Survey of All Aspects of This Group of Diseases From a Global Standpoint*, Vol. I. W. B. Saunders, Philadelphia.

CHRISTENSEN, B. M., 1986 Immune mechanisms and mosquito-filarial worm relationships, pp. 145–160 in *Immune Mechanisms in Invertebrate Vectors*, edited by A. M. LACKIE. Clarendon Press, Oxford.

CHRISTENSEN, B. M., and D. R. SUTHERLAND, 1984 *Brugia pahangi:* exsheathment and midgut penetration in *Aedes aegypti*. Trans. Am. Microsc. Soc. **103:** 423–433.

DENNHÖFER, U., 1971 Erblichkeit der Ubertragungsfähigkeit bzw. Resistenz gegen Vogelmalaria bei der Stechmücke *Culex pipiens*. Anz. Schädlingskd. **44:** 84–91.

FATOKUN, D. A., D. I. MENANCIO-HAUTEA, D. DANESH and N. D. YOUNG, 1992 Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. Genetics **132:** 841–846.

FRIZZI, G., A. RINALDI and U. BIANCHI, 1975 Genetic studies on mechanisms influencing the susceptibility of anopheline mosquitoes to plasmodial infection. Mosq. News **35:** 505–508.

GILCHRIST, B. M. and J. B. S. HALDANE, 1947 Sex linkage and sex determination in a mosquito, *Culex molestus*. Hereditas **33:** 175–190.

GRAVES, P. M., and C. F. CURTIS, 1982 Susceptibility of *Anopheles gambiae* to *Plasmodium yoelii nigeriensis* and *Plasmodium falciparum*. Ann. Trop. Med. Parasitol. **76:** 633–639.

HUFF, C. G., 1929 The effects of selection upon susceptibility to bird malaria in *Culex pipiens* Linn. Ann. Trop. Med. Parasitol. **23:** 427–442.

HUFF, C. G., 1931 The inheritance of natural immunity to *Plasmodium cathemerium* in two species of *Culex*. J. Prev. Med. **5:** 249–259.

KILAMA, W. L., 1973 Distribution of a gene for susceptibility to *Plasmodium gallinaceum* in populations of *Aedes aegypti* (L.). J. Parasitol. **59:** 920–924.

KILAMA, W. L., and G. B. CRAIG, JR., 1969 Monofactorial inheritance of susceptibility to *Plasmodium gallinaceum* in *Aedes aegypti*. Ann. Trop. Med. Parasitol. **63:** 419–432.

KOSAMBI, D. D., 1944 The estimation of map distance from recombination values. Ann. Eugen. **12:** 172–175.

LANDER, E. S., P. GREEN, J. ABRAMHAMSON, A. BARLOW, M. DALY *et al.*, 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

LINCOLN, S. E., and E. S. LANDER, 1990 Mapping Genes Controlling Quantitative Traits Using MAPMAKER/QTL. Whitehead Institute for Biomedical Research, Cambridge, MA.

MATTHEWS, T. C., and G. B. CRAIG, JR., 1989 Isozyme polymorphisms maintained by lethal loci in inbred strains of *Aedes triseriatus*. J. Hered. **80:** 53–57.

MATTHEWS, T. C., and MUNSTERMANN, L. E., 1994 Chromosomal repatterning and linkage group conservation in mosquito karyotypic evolution. Evolution **48:** 146–154.

MITCHELL, C. J., 1983 Mosquito vector competence and arboviruses, pp. 63–92 in *Current Topics in Vector Research*, vol. I, edited by K. F. HARRIS. Praeger Publishing, New York.

MUNSTERMANN, L. E., 1990 Linkage map of the yellow fever mosquito, *Aedes aegypti*, pp. 179–183 in *Genetic Maps*, vol. 3, Ed. 5, edited by S. J. O'BRIEN. Cold Spring Harbor Press, Cold Spring Harbor, NY.

MUNSTERMANN, L. E., 1994 Unexpected genetic consequences of colonization and inbreeding: allozyme tracking in Culicidae (Diptera). Ann. Entomol. Soc. Am. **87:** 157–164.

SAS Institute, 1990 *SAS Language Guide for Personal Computers*. SAS Institute, Cary, NC.

SEVERSON, D. W., 1994 Applications of molecular marker analysis to mosquito vector competence. Parasitol. Today **10:** 336–340.

SEVERSON, D. W., A. MORI, Y. ZHANG and B. M. CHRISTENSEN, 1993 Linkage map for *Aedes aegypti* using restriction fragment length polymorphisms. J. Hered. **84:** 241–247.

SEVERSON, D. W., A. MORI, Y. ZHANG and B. M. CHRISTENSEN, 1994a The suitability of restriction fragment length polymorphism markers for evaluating genetic diversity among and synteny between mosquito species. Am. J. Trop. Med. Hyg. **50:** 425–432.

SEVERSON, D. W., A. MORI, Y. ZHANG and B. M. CHRISTENSEN, 1994b Chromosomal mapping of two loci affecting filarial worm susceptibility in *Aedes aegypti*. Insect Mol. Biol. **3:** 67–73.

SEVERSON, D. W., A. MORI, V. A. KASSNER and B. M. CHRISTENSEN, 1994c Comparative linkage maps for the mosquitoes, *Aedes albopictus* and *Aedes aegypti*, based on common RFLP loci. Insect Mol. Biol. (in press).

STAM, P., 1993 Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. **3:** 739–744.

TABACHNICK, W. J., G. P. WALLIS, T. H. G. AITKEN, B. R. MILLER, G. D. AMATO *et al.*, 1985 Oral infection of *Aedes aegypti* with yellow fever virus: geographic variation and genetic considerations. Am. J. Trop. Med. Hyg. **34:** 1219–1224.

THATHY, V., D. W. SEVERSON and B. M. CHRISTENSEN, 1994 Reinterpretation of the genetics of susceptibility of *Aedes aegypti* to *Plasmodium gallinaceum*. J. Parasitol. **80:** 705–712.

VAN OOIJEN, J. W., 1992 Accuracy of mapping quantitative trait loci in autogamous species. Theor. Appl. Genet. **84:** 803–811.

WARBURG, A., and L. H. MILLER, 1991 Critical stages in the development of *Plasmodium* in mosquitoes. Parasitol. Today **7:** 179–181.

WARD, R. A., 1963 Genetic aspects of the susceptibility of mosquitoes to malarial infection. Exp. Parasitol. **13:** 328–341.

WATERS, A. P., D. G. HIGGINS and T. F. MCCUTCHAN, 1991 *Plasmodium falciparum* appears to have arisen as a result of lateral transfer between avian and human hosts. Proc. Natl. Acad. Sci. USA **88:** 3140–3144.

WING, R. A., H. B. ZHANG and S. D. TANKSLEY, 1994 Map-based cloning in crop plants. Tomato as a model system: I. Genetic and physical mapping of *jointless*. Mol. Gen. Genet. **242:** 681–688.

Communicating editor: Z-B. ZENG