

Dynamics of Molecular Markers Linked to the Resistance Loci in a Mosquito-Plasmodium System

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ABSTRACT

Models on the evolution of resistance to parasitism generally assume fitness tradeoffs between the costs of being parasitized and the costs associated with resistance. This study tested this assumption using the yellow fever mosquito *Aedes aegypti* and malaria parasite *Plasmodium gallinaceum* system. Experimental mosquito populations were created by mixing susceptible and resistant strains in equal proportions, and then the dynamics of markers linked to loci for Plasmodium resistance and other unlinked neutral markers were determined over 12 generations. We found that when the mixed population was maintained under parasite-free conditions, the frequencies of alleles specific to the susceptible strain at markers closely linked to the loci for resistance (QTL markers) as well as other unlinked markers increased significantly in the first generation and then fluctuated around equilibrium frequencies for all six markers. However, when the mixed population was exposed to an infected blood meal every generation, allele frequencies at the QTL markers for resistance were not significantly changed. Small population size caused significant random fluctuations of allele frequencies at all marker loci. Consistent allele frequency changes in the QTL markers and other unlinked markers suggest that the reduced fitness in the resistant population has a genome-wide effect on the genetic makeup of the mixed population. Continuous exposure to parasites promoted the maintenance of alleles from the resistant Moyo-R strain in the mixed population. The results are discussed in relation to the proposed malaria control strategy through genetic disruption of vector competence.

HOST populations often exhibit considerable polymorphism in resistance to parasites despite ubiquitous parasites often having deleterious effects on host reproductive success. Conceptual and mathematical models on the evolution of resistance generally assume fitness tradeoffs between the costs of being parasitized and the costs associated with resistance (RENAUD and DE MEEUS 1991; FRANK 1993; RENAUD *et al.* 1996; BOOTS and HARAGUCHI 1999; SASAKI and GODFRAY 1999; SASAKI 2000). This assumption is based on the argument that if no fitness costs are associated with resistance, then parasite-driven selection would have pushed the host population to complete fixation of the resistance genes, and thus there should be no heritable variation in resistance. Indirect empirical evidence supports the hypothesis that resistance to parasites is costly (MICHALAKIS and HOCHBERG 1994; KRAAIJEVELD and GODFRAY 1997; YAN *et al.* 1997; FELLOWES *et al.* 1998; WEBSTER and WOOLHOUSE 1999; MORET and SCHMID-HEMPEL 2000; RIGBY *et al.* 2002). For example, when *Drosophila melanogaster* adults were selected for increased resistance to parasitoid wasps in the laboratory, the competitive abil-

ity of the larvae was reduced (KRAAIJEVELD and GODFRAY 1997). The reduced fitness in resistant fly larvae is probably due to reallocation of limiting resources from trophic to defense functions, because the resistant fly larvae had twice as many haemocytes, the primary cellular immune defense against parasitoids, as the control flies (KRAAIJEVELD *et al.* 2000).

Negative correlation between resistance and fitness could result from the pleiotropic effects of the resistance genes or from linkage disequilibrium between resistance genes and genes determining host fitness. Usually only those resulting from pleiotropy are considered true costs (STOWE 1998), because in outbreeding species genetic correlation resulting from linkage disequilibrium is expected to decay over time with recombination (LEWONTIN 1974). Therefore, correlation that results from linkage disequilibrium does not represent a permanent constraint on the evolution of resistance. Traditional approaches for examining the costs associated with resistance to parasites involve selection of resistant and susceptible populations and comparisons of fitness between the two populations in either the absence or the presence of parasites (*e.g.*, YAN *et al.* 1997). Such approaches cannot discriminate pleiotropic effects of the resistance genes from the effects of population life history and genetic correlation resulting from linkage disequilibrium. An alternative approach is to examine the dynamic behavior of resistance genes and linkage

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disequilibrium between markers linked and unlinked to the resistance loci in populations polymorphic for the resistance phenotype. This approach may provide valuable insight into the role of linkage disequilibrium on fitness reduction in parasite-resistant populations.

The goal of this study is to determine the dynamics of molecular markers linked to loci conferring *Plasmodium gallinaceum* resistance when a susceptible *Aedes aegypti* mosquito population is mixed with a resistant population. Resistance refers to the inability of malaria parasites to develop to the oocyst stage on the mosquito midgut, a common, naturally occurring parasite-inhibiting mechanism (KILAMA and CRAIG 1969; THATHY *et al.* 1994; SEVERSON *et al.* 1995). Here, we use the term "resistance," which is distinct from another mechanism wherein melanotic encapsulation of the parasite is involved (COLLINS *et al.* 1986). *A. aegypti* resistance to *P. gallinaceum* is determined by at least two loci (SEVERSON *et al.* 1995). The major quantitative trait locus (QTL) on chromosome 2 accounted for 49–65% of the observed phenotypic variance while a minor QTL on chromosome 3 explained 10–14% of the phenotypic variance. Both QTL exhibited a partial dominance effect on susceptibility, wherein the dominance effect was derived from the refractory parent. No significant epistasis between these QTL was detected. Recent studies suggest that at least another minor QTL may be involved (D. W. SEVERSON, unpublished data). The resistance genes have not yet been cloned.

YAN *et al.* (1997) compared the reproductive success and survivorship of two *A. aegypti* populations, resistant (Moyo-R strain) and susceptible (Moyo-S strain) to *P. gallinaceum*. The two populations were selected from a common stock population through inbreeding (inbreeding coefficient = 0.5; THATHY *et al.* 1994). The resistant population had a significantly shorter larva-to-adult developmental time and a smaller body size, took a smaller blood meal, and subsequently laid fewer eggs than the susceptible population. The mean longevity of the resistant population was nearly one-half of the susceptible population. Thus, the resistant population exhibited substantially reduced fitness compared to the susceptible population under the laboratory conditions. The mechanisms for fitness reduction in the resistant population are not clear. Potential factors include variation in inbreeding depression among the population founders, founder effects, the pleiotropic effects of the resistance genes, or linkage disequilibrium between resistance genes and genes determining host fitness.

If the reduced fitness observed in the resistant mosquito population results from the pleiotropic effects of resistance genes, one would expect that the resistant-strain-specific allele frequencies for the markers closely linked to the loci (QTL markers) for resistance should decrease gradually in a population polymorphic for resistance, while the frequencies of other markers unlinked to the resistance loci remain unchanged. This

study tested this prediction. We also examined how parasite infection and genetic drift affected the dynamics of QTL marker allele frequencies. Several studies have demonstrated reduced fecundity in *P. gallinaceum*-infected *A. aegypti* mosquitoes (HACKER and KILAMA 1974; FREIER and FRIEDMAN 1976). Under laboratory conditions, *P. gallinaceum* infection reduced fecundity but did not significantly affect the survivorship of *A. aegypti* (YAN *et al.* 1997).

MATERIALS AND METHODS

Mosquitoes and parasites: Two *A. aegypti* strains, Moyo-R and Red, were used in this study. The Moyo-R population is highly resistant to *P. gallinaceum*, and Red is highly susceptible. The two strains were previously used for genetic mapping of Plasmodium resistance (SEVERSON *et al.* 1995). Moyo-R and Red strains were used for this study because strain-specific molecular markers linked to the loci conferring resistance were available so that the dynamics of strain-specific alleles could be tracked in artificial populations created by mixing the two strains. The main goal of this study was to determine the dynamics of alleles specific to the resistant or susceptible strains and compare how the dynamics in allele frequency vary among the markers linked or unlinked to the Plasmodium-resistant QTL. Differences in the relative fitness and inbreeding level between Moyo-R and Red strains would have similar effects on all markers. Unless otherwise stated, mosquito larvae were reared under standard insectary conditions (YAN *et al.* 1997). Adult mosquitoes were maintained in an environmental chamber regulated at $26.5^{\circ} \pm 0.5^{\circ}$ and $80 \pm 5\%$ room humidity under a 16-hr light:8-hr dark light cycle. Four- to 5-day-old female mosquitoes were deprived of sucrose for 24 hr before receiving an infected or uninfected blood meal.

Maintenance of the *P. gallinaceum* parasite was as previously described (THATHY *et al.* 1994). Female mosquitoes were allowed to engorge on restrained White Leghorn chicks (Northern Hatchery, Beaver Dam, WI). Parasite-infected chicks were obtained by sporozoite transmission. The parasitemia (percentage of infected red blood cells) and percentage of gametocytes were monitored daily beginning 1 week postinfection. When the parasitemia of an infected chick reached at least 10% with >0.5% gametocyte development, the naive mosquitoes were allowed to blood feed for 15 min. Following blood feeding, fully engorged mosquitoes were separated and thereafter continuously supplied with sucrose-saturated pads. Mosquito susceptibility was examined by dissecting individual mosquitoes 6–7 days post-blood feeding and scoring the number of oocysts that had developed on the midgut.

Mixing susceptible with resistant mosquito populations: Three experimental populations were created by mixing the susceptible Red strain and resistant Moyo-R strain, and the main characteristics of the three populations are summarized in Table 1. Population 1 was created by mixing 500 individuals of Moyo-R and 500 Red individuals and was maintained in a cage of size $45 \times 45 \times 45$ cm³. The population was allowed to blood feed on an uninfected chicken for 30 min each generation. Eggs were collected and reared to adults under standard insectary conditions (YAN *et al.* 1997). To minimize intraspecific competition, a maximum of ~300 first instar larvae were reared in trays containing 3 liters of water. About 1000 pupae were reared to adults, and adults 5–7 days post-emergence were allowed to blood feed on an uninfected chicken. This process was repeated for a total of 12 generations. Thus, the major factors determining the fitness of the

TABLE 1
Characteristics of the three experimental populations

Population	Population size	Exposure to <i>P. gallinaceum</i>	RFLP genotyping
1	1000	No	Every generation
2	1000	Yes	Every three generations
3	20	No	Every three generations

The populations were created by mixing a *P. gallinaceum*-susceptible *A. aegypti* population (Red strain) and a highly resistant population (Moyo-R strain) in equal proportions. The populations were cultured for a total of 12 generations in the laboratory.

two strains were the ability of female adults to obtain blood meal, blood meal size and fecundity, and male mating success. The ability of female adults to obtain a blood meal and blood meal size may be density dependent. Population 2 was maintained under the same condition as population 1 except that all mosquitoes were exposed to *P. gallinaceum*-infected chickens at each generation. Population 3 was initiated with only 10 individuals per strain in a cage of $20 \times 20 \times 20$ cm³, maintained at a population size of 20 individuals at later generations to test the effect of genetic drift, and blood fed on uninfected chickens. Equal numbers of male and female mosquitoes within each strain were used to initiate the populations. Individual colonies of Red and Moyo-R strains were also maintained for 12 generations under the same conditions as population 1. We recognized the shortfall of using a single population for each treatment; however, the genotyping work involved for each population (see below) was very labor intensive, and genotyping replicating populations was not feasible.

We monitored the susceptibility of the mixed populations to *P. gallinaceum* every three generations. We infected 80–100 individuals per population and thereafter dissected 50 individuals to determine susceptibility. The Red and Moyo-R strains were also exposed to the same infected chickens and later dissected as controls.

Restriction fragment length polymorphism and probe selection: Genomic DNA extraction from individual mosquitoes, digestion with *EcoRI*, Southern blotting, and hybridization were as previously described (SEVERSON 1997). Six mapped restriction fragment length polymorphism (RFLP) markers were used to genotype mosquitoes (Figure 1). Markers LF98 and LF409 are within a major QTL for *P. gallinaceum* susceptibility on chromosome 2, while marker *Mall* is linked with the

minor QTL on chromosome 3 (SEVERSON *et al.* 1995). Marker ARC1 is linked with a second major effect QTL located on the opposite arm of chromosome 2 (D. W. SEVERSON, unpublished data). The other two markers (LF178 and LF198) are not linked to any known QTL for *P. gallinaceum* susceptibility. All clones used were random cDNA clones with unknown functions, except *Mall*, which is a known gene (JAMES *et al.* 1989). The Red and Moyo-R populations exhibit strain-specific RFLP patterns and do not share any restriction fragments for LF178, ARC1, and *Mall* (Figure 2), but share one fragment for LF98 and LF198. For LF409, the Red strain carries one unique allele and Moyo-R did not have any unique allele due to allele sharing with Red. For population 1, 50 adults were genotyped with the six markers every generation except generation 2 (Southern blots of this generation failed). For populations 2 and 3, 50 adults were randomly chosen and genotyped with the same six markers every 3 generations. Moyo-R and Red strains were genotyped at generations 0, 6, and 12 to determine whether allele frequencies changed within single-strain mosquito cultures.

Data analysis: Allele frequency and Hardy-Weinberg equilibrium test: RFLP genotype data were analyzed using the GENEPOP computer program (RAYMOND and ROUSSET 1995). Analyses included computation of allele frequencies, observed and expected heterozygosity per locus, and a test for conformance with Hardy-Weinberg equilibrium (HWE) at each locus, using the exact test (WEIR 1990; GUO and THOMPSON 1992). We further tested whether distortion from HWE resulted from deficient or excessive heterozygosity, using the method described by ROUSSET and RAYMOND (1995). Conformance with HWE was tested by using the probability test for each locus, for each population at each generation, using the GENEPOP computer program. RFLP allele frequency differences between the starting population and subsequent generations were tested using Fisher's exact test (GOUDET 1995).

Linkage disequilibrium: Linkage disequilibrium was tested for all pairs of RFLP loci, within the same chromosomes or between different chromosomes, to evaluate the independence of genotypes at one locus relative to all other loci. The correlation coefficient between alleles at different loci was used to express the magnitude of linkage disequilibrium and was computed on the basis of the procedure of WEIR and COCKERHAM (1984), using the LINKDOS computer program available in the GENEPOP computer program package. Fisher's exact test was used to test the significance of linkage disequilibrium.

Susceptibility to *Plasmodium* infection: Because mosquitoes were exposed to infected chickens with different parasitemias at different generations, comparisons of mosquito susceptibility between generations is not appropriate. However, comparisons among populations within the same generation are appropriate because all mosquitoes within generations blood fed on the same infected chicken. Among-population variation in infection intensity was analyzed using the Wilcoxon rank-

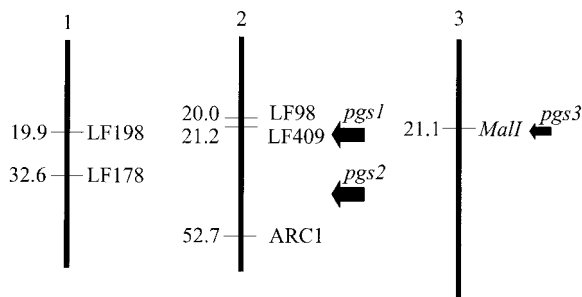


FIGURE 1.—Relative map position of the six *A. aegypti* ($2N = 6$) RFLP markers used in the study. Map distances are in Kosambi centimorgans. The three QTL for the susceptibility of *A. aegypti* to *P. gallinaceum* are indicated (*pgs1*, *pgs2*, and *pgs3*). *pgs1* and *pgs2* on chromosome 2 are the major QTL while *pgs3* on chromosome 3 is the minor QTL (SEVERSON *et al.* 1995; D. W. SEVERSON, unpublished data).

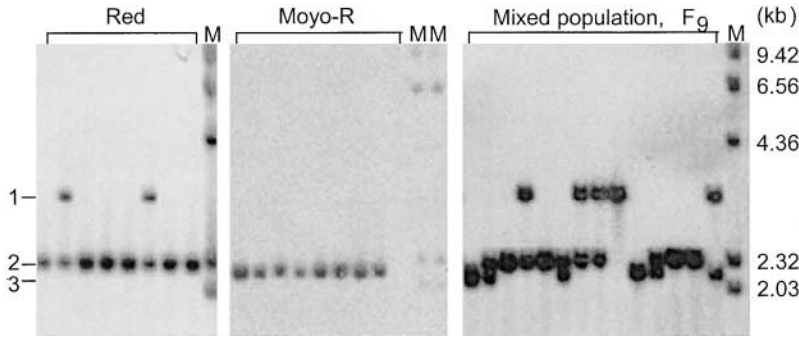


FIGURE 2.—A representative Southern blot autoradiograph for Moyo-R and Red strains and a mixed population (population 2, generation 9) probed with marker ARC1. Alleles 1 and 2 are specific to the Red strain, and allele 3 is specific to the Moyo-R strain. The mixed population exhibited segregation of all three alleles. M is the molecular size marker.

sum test, and prevalence was analyzed using the χ^2 test (SAS 1994). Infection intensity is defined as the number of oocysts in an infected individual, and prevalence as the percentage of infected individuals after exposure to an infected blood meal.

RESULTS

Allele frequency changes and the HWE test: Allele frequencies within the Moyo-R and Red strains did not change significantly at any of the six loci during single-strain colony maintenance over the 12 generations (data not shown). For population 1, the frequencies of alleles specific to the susceptible Red strain increased significantly from the initial values for the markers linked with QTL (LF98, LF409, ARC1, and *Mall*) and also the two unlinked markers at generation 1 ($P < 0.001$). At later generations allele frequencies fluctuated, but were generally significantly higher than the initial frequencies (Figure 3). The opposite pattern was observed for alleles specific to the resistant Moyo-R strain for all six markers. Increases in Red-specific allele frequencies and decreases in Moyo-R-specific allele frequencies for markers linked with known QTL for susceptibility and also unlinked markers suggest that the reduced fitness of the Moyo-R strain has a genome-wide effect on the genetic makeup of the mixed population in a parasite-free environment.

For population 2, the allele frequencies of three markers (LF98, LF198, and *Mall*) did not change significantly from the initial values at generations 3, 6, 9, and 12 ($P > 0.05$; Figure 3). The remaining three markers (LF178, ARC1, and LF409) exhibited significant increases in the frequencies of the Red-specific alleles at generations 3, 6, and 9, but returned to the initial frequency levels at generation 12. In comparison with population 1, the Red-specific allele frequencies of population 2 were generally lower, suggesting that exposure to a *Plasmodium*-infected blood meal generally enabled Moyo-R-specific alleles to be maintained in the population.

For population 3, allele frequencies at each of the six marker loci were more variable than those observed with populations 1 or 2, with the frequencies of the Red-specific alleles being higher than the initial values for three markers (LF198, LF178, and LF409) but lower

than those for the other three markers (LF98, *Mall*, and ARC1; Figure 3). The allele frequency changes in all six markers in population 3 were more dramatic than those in population 1, suggesting that random drift due to the small population size of the founders played an important role in allele dynamic behaviors.

The genotypes of all six markers were in HWE for population 1 (Table 2). For population 2, only one marker (LF409) at generation 6 was not in HWE. However, this is within the acceptable type I error rate for multiple chi-square tests, suggesting that the population was random mating. For population 3, five markers at various generations (37.5% of the Hardy-Weinberg tests) were in Hardy-Weinberg disequilibrium, mostly due to a deficiency of heterozygotes (Table 2).

Linkage disequilibrium: Linkage disequilibrium was analyzed for all pairs of markers both on the same chromosome and between chromosomes (Table 3). LF198-LF178 on chromosome 1 and LF409-LF98 on chromosome 2 exhibited significant linkage disequilibrium in the Red parent strain. For populations 1 and 3, the observed linkage disequilibrium decayed, but remained statistically significant in most generations. Overall 50 and 25% of the marker pairs on the same chromosome were in linkage disequilibrium for populations 1 and 3, respectively, but only 6.25% of the marker pairs for population 2 (Table 3). Fewer marker pairs between chromosomes were in linkage disequilibrium (13.6% for population 1, 6.8% for population 2, and 15.9% for population 3) than that observed for markers pairs on the same chromosome.

Population susceptibility to *Plasmodium* infection: All three populations exhibited much higher infection intensities and prevalence ($P < 0.001$) at all generations that we tested than did the resistant Moyo-R parent strain (Table 4). The decrease in the mean infection intensity in the Red strain from generation 3 to 12 was likely due to variability in infectivity of individual chickens and not due to genetic changes in the strain, because it exhibited no significant changes in allele frequencies at any of the six marker loci over the 12 generations. Population susceptibility can be compared only among the three populations and between the two single-strain colonies within the same generation. The three popula-

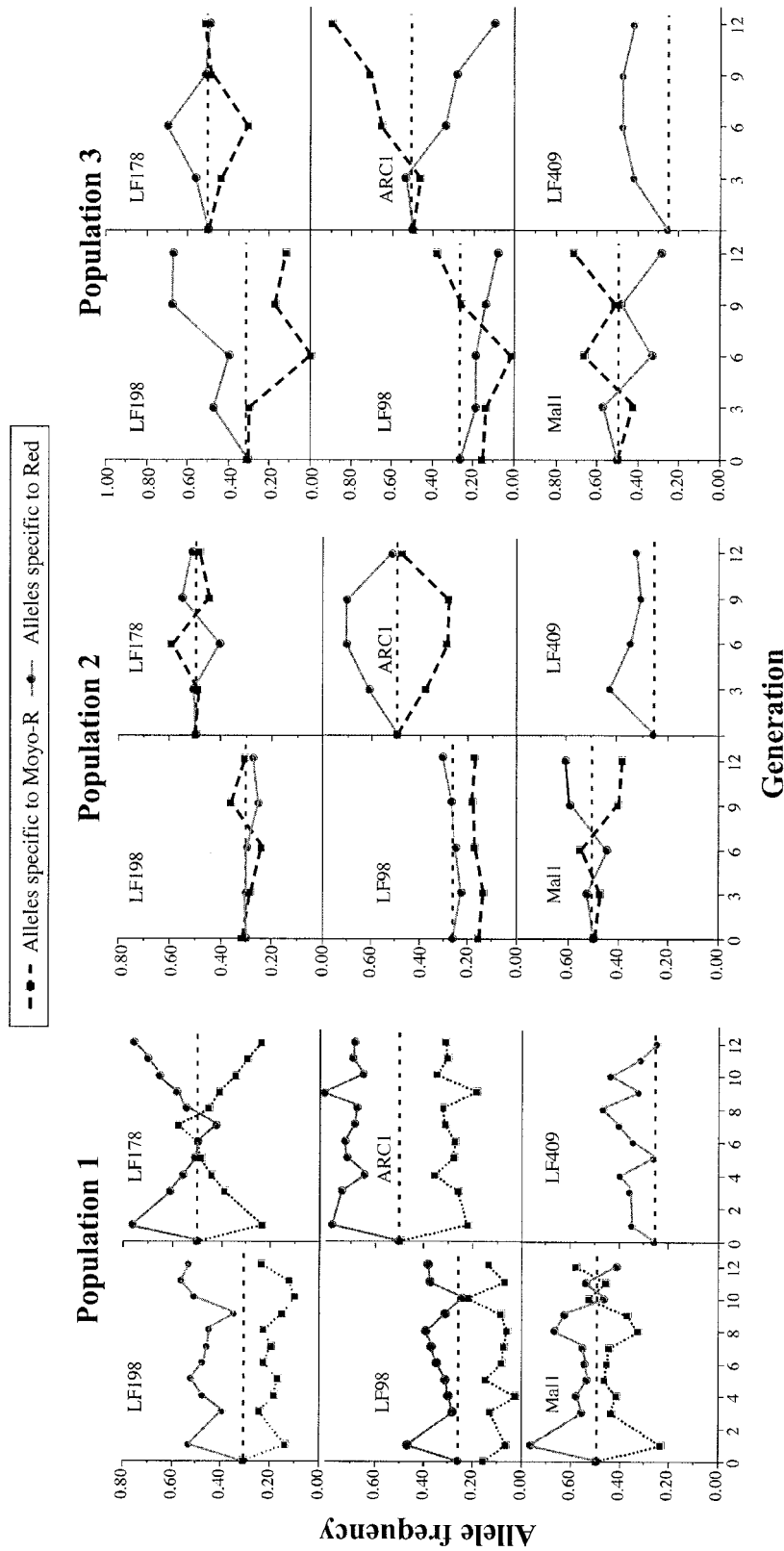


FIGURE 3.—Dynamics of allele frequencies at the six RFLP marker loci. For marker LF409, the Red strain had a unique allele and Moyo-R did not have any unique allele due to allele sharing with Red; thus only the frequencies of the Red-unique allele are presented. See Table 1 for detailed description of the three populations.

TABLE 2
Observed heterozygosity and HWE test results for the three *A. aegypti* populations

Locus	Population 1 generation				Population 2 generation				Population 3 generation			
	3	6	9	12	3	6	9	12	3	6	9	12
LF198	0.605	0.625	0.739	0.563	0.691	0.574	0.659	0.609	0.429 ^{*,a}	0.744 ^{***,b}	0.600	0.540 ^{*,b}
LF178	0.540	0.598	0.478	0.522	0.685	0.776	0.511	0.545	0.692	0.947 ^{***,b}	0.725	0.816 ^{*,b}
ARC1	0.548	0.621	0.478	0.708	0.589	0.633	0.617	0.542	0.524	0.585	0.500	0.160
LF409	0.524	0.478	0.435	0.367	0.571	0.339 ^{*,a}	0.447	0.372	0.667 ^{*,b}	0.400	0.650	0.680 ^{*,b}
LF98	0.500	0.563	0.500	0.455	0.527	0.567	0.553	0.581	0.436 ^{*,a}	0.410	0.600	0.640
<i>Mall</i>	0.415	0.598	0.391	0.458	0.618	0.536	0.468	0.568	0.683	0.500	0.667 ^{*,b}	0.531

See Table 1 for description of the three populations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^aIndicates heterozygote deficit over that expected from HWE.

^bIndicates a heterozygote excess.

tions had significantly lower infection intensities than the susceptible Red strain had at generations 3 and 6 ($P < 0.01$ for all tests). Infection intensities at generations 9 and 12 with populations 1 and 3 were similar to that of the Red strain ($P > 0.05$), but population 2 harbored significantly fewer parasites than did the Red strain ($P < 0.05$). Susceptibility of population 2 was similar to population 1 at generations 3 and 6, but significantly lower infection intensities were observed at generations 9 and 12 ($P < 0.05$; Table 4).

DISCUSSION

In this study, we observed that when a Plasmodium-susceptible population was mixed with a resistant population in equal proportions and maintained under parasite-free conditions (population 1), the frequencies of alleles specific to the susceptible population increased significantly from the initial frequencies in the first generation for four marker loci linked with QTL for Plasmodium susceptibility and also for two marker loci unlinked to the QTL loci. The frequencies of Red-specific alleles then fluctuated around equilibrium frequencies in later generations. Similarly, the frequencies of Moyo-R-specific alleles decreased significantly from the initial frequencies observed at five marker loci. Significant and consistent changes in allele frequency in all six markers in this population have not likely occurred by chance. Allele frequency changes in markers linked with QTL and also in markers unlinked to the QTL suggest that the reduced fitness observed in the Moyo-R population has a genome-wide effect on the genetic makeup of the mixed population.

The genome-wide effect on allele frequencies in the mixed population may be caused by linkage disequilibrium, but not assortative mating between Red or Moyo-R individuals, because all markers were in Hardy-Weinberg equilibrium (population 1 in Table 1). Further, data on population susceptibility to the Plasmodium parasite are consistent with the observed result that the

frequencies of susceptible alleles increased in the mixed population, because Plasmodium susceptibility of this population was similar to the Red strain after nine generations of culture (Table 4). If resistant loci were associated with a large fitness cost, one would expect that the markers linked to the resistant QTL (LF98, LF409, and *Mall*) should show more dramatic changes in allele frequencies over time than other markers not linked to the resistant QTL (LF198 and LF178). That gradual changes in Moyo-R- or Red-specific allele frequencies were not observed and the dynamics of the alleles specific to the Red strain were similar for all six markers suggests that the resistant loci are not associated with a large fitness effect. Other factors, including inbreeding depression in the resistant strain and genetic background difference, may have contributed to the substantial fitness difference between Moyo-R and Moyo-S strains reported earlier (YAN *et al.* 1997). The hypothesis that linkage disequilibrium may have caused allele frequency changes for markers linked and unlinked to the Plasmodium-resistant loci needs further investigation using empirical and theoretical approaches.

Our results suggest that continuous exposure to parasites promoted the maintenance of alleles from the resistant Moyo-R population in the mixed population (population 2; Figure 3). Population susceptibility data were consistent with the allele frequency data. That is, this population exhibited significantly lower infection intensities in later generations than the mixed population that was never exposed to the parasite during the 12-generation laboratory culture period (Table 4). This phenomenon may be partially explained by fitness reduction induced by the parasite. Previous studies demonstrated that parasite infection reduced egg production by 17.5% in the *Anopheles gambiae*/*P. falciparum* system (HOGG and HURD 1997) and resulted in a 38–48% reduction in fecundity in the *A. stephensi*/*P. yoelii* system (JAHAN and HURD 1997). However, the effect of reduced quality of the infected blood meal on mosquito fecundity was not considered in these studies. *P. gallinaceum* infec-

TABLE 3
Linkage disequilibrium among marker loci across the three populations

Generation	Population 1				Population 2			
	3	6	9	12	3	6	9	12
Within chromosomes								
LF198-LF178	0.235**	0.130*	0.161	0.386**	0.109	0.091	0.142	0.072
ARC1-LF409	0.158	0.133	0.152	0.169	0.202	0.101	0.205	0.198
ARC1-LF98	0.194*	0.137	0.189*	0.056	0.055	0.147	0.144	0.136
LF409-LF98	0.364**	0.175*	0.276*	0.184	0.094	0.310***	0.091	0.194
Between chromosomes								
LF198-ARC1	0.142	0.213**	0.179	0.143	0.045	0.164	0.092	0.121
LF198-LF409	0.122	0.111	0.167	0.229	0.022	0.135	0.207	0.073
LF198-LF98	0.095	0.120	0.169*	0.299	0.056	0.092	0.148	0.156
LF178-ARC1	0.122	0.085	0.158	0.143	0.173*	0.081	0.142	0.151
LF178-LF409	0.112	0.051	0.320***	0.529*	0.053	0.080	0.003	0.196
LF178-LF98	0.089	0.050	0.164**	0.231	0.138	0.102	0.112	0.165
LF198- <i>Mall</i>	0.065	0.136	0.013	0.169	0.073	0.068	0.173	0.092
LF178- <i>Mall</i>	0.072	0.151**	0.075	0.178	0.123	0.138	0.085	0.238*
ARC1- <i>Mall</i>	0.075	0.061	0.163	0.260	0.132	0.141	0.239**	0.074
LF409- <i>Mall</i>	0.088	0.043	0.083	0.341	0.207	0.082	0.100	0.189
LF98- <i>Mall</i>	0.055	0.079	0.079	0.237	0.122	0.066	0.159	0.149
Population 3								
Generation	3	6	9	12	Red: 0	Moyo-R: 0		
Within chromosomes								
LF198-LF178	0.158*	0.187	0.375***	0.205	0.235*	0.011		
ARC1-LF409	0.169	0.127	0.166	0.219	0.080	—		
ARC1-LF98	0.133	0.319	0.132	0.039	0.088	—		
LF98- LF409	0.381*	0.207	0.403**	0.483**	0.896***	—		
Between chromosomes								
LF198-ARC1	0.149	0.026	0.216	0.274**	0.004	—		
LF198- LF409	0.068	0.456	0.155	0.044	0.009	—		
LF198-LF98	0.131	0.150	0.176	0.087	0.066	0.006		
LF178-ARC1	0.144	0.091	0.207***	0.027	0.030	—		
LF178-LF409	0.064	0.303*	0.123	0.159	0.058	—		
LF178-LF98	0.113	0.127	0.169	0.175	0.028	0.027		
LF198- <i>Mall</i>	0.235*	0.134	0.121	0.089	0.072	—		
LF178- <i>Mall</i>	0.819***	0.272	0.272***	0.125	0.055	—		
ARC1- <i>Mall</i>	0.157	0.064	0.222**	0.062	0.072	—		
LF409- <i>Mall</i>	0.059	0.145	0.164	0.292	0.089	—		
LF98- <i>Mall</i>	0.117	0.183	0.161	0.221	0.087	—		

Correlation coefficients between alleles of different loci are reported. —, the correlation coefficient could not be calculated due to monomorphism of one or two markers. See Table 1 for description of the three populations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tion reduced fecundity by 9% in susceptible *A. aegypti* individuals after the effect of reduced protein contents in the infected blood meal was taken into consideration (YAN *et al.* 1997). Under conditions where mosquito populations are exposed to the parasite, the fitness reduction in fecundity in the susceptible strain may have counterbalanced the fitness reduction associated with Plasmodium resistance and resulted in the maintenance of Moyo-R-specific alleles in the population.

Despite the apparent strong fitness costs associated with resistance to Plasmodium parasites (YAN *et al.*

1997), natural mosquito populations exhibit high polymorphism for susceptibility to *P. gallinaceum*. For example, KILAMA (1973) studied susceptibility of seven African *A. aegypti* populations to *P. gallinaceum* and found that 10–30% of the mosquitoes did not harbor oocysts. High polymorphism in parasite susceptibility can be explained by three hypotheses. The first is that polymorphism in susceptibility is determined by fitness tradeoffs between the costs of maintaining resistance mechanisms irrespective of the presence of parasites and costs of mounting immune reactions and the costs of being infected.

TABLE 4

Mean infection intensities of the three *A. aegypti* populations and the two parent populations used to create the mixed populations (Moyo-R and Red strains)

Generation	Population 1 (%)	Population 2 (%)	Population 3 (%)	Red (%)	Moyo-R (%)
0				93.6 (94.0)	2.3 (8.0)
3	24.6 (90.0)	39.7 (92.0)	23.5 (84.0)	—	—
6	34.6 (84.0)	41.8 (93.7)	20.5 (91.4)	50.8 (100)	—
9	23.5 (88.4)	12.8 (68.0)	22.6 (94.4)	23.3 (100)	—
12	16.2 (63.3)	11.1 (70.0)	9.6 (65.4)	15.9 (96.1)	1.6 (5.4)

The number in parentheses is the percentage of prevalence of infection. —, the trait was not measured.

This hypothesis predicts that the mosquito populations should be more susceptible to the parasite in areas where malaria is less prevalent. The second hypothesis is that selection pressure by the malaria parasite is low in nature. Plasmodium infection rates in mosquito vectors are usually <10% even in areas highly endemic for malaria (TAYLOR *et al.* 1990; PAUL *et al.* 1995). The third possibility is dynamic coevolution between the parasite and the mosquito host wherein mosquito resistance to malaria parasite infection is strain specific or depends on the history (*e.g.*, sympatry or allopatry) of the host-parasite interactions (MORAND *et al.* 1996). That is, if there are multiple strains of parasites and resistance to one strain does not confer resistance to other strains, then allele frequencies at resistance loci are expected to oscillate over time. The parasites that we used were from a single strain, and thus oscillation of allele frequencies is not expected. Further studies are needed to determine the importance of each mechanism in shaping polymorphisms in mosquito susceptibility to malaria parasites in nature.

Malaria disease due to the Plasmodium parasite is one of the most important parasitic diseases today, with ~500 million clinical cases each year, including ~2.7 million deaths, mainly among children. The emergence of pesticide resistance in mosquitoes and antimalarial drug resistance in Plasmodium has significantly limited malaria control efforts. Efforts toward vaccine development show little promise for disease control in the near future. One proposed strategy is to control malaria transmission on the basis of genetic disruption of mosquito vector competence (COLLINS and BESANSKY 1994; JAMES *et al.* 1999). The success of genetic control strategies depends on the introduction and spread of genes for resistance into natural vector populations. Our results provide general support for the concept that the simple release of Plasmodium resistance genes into natural populations, via transgenic mosquitoes, is not a likely strategy for significantly influencing allele frequencies in existing populations, but instead is dependent on some type of gene-driving mechanism. If active transposable elements are used to drive genes into natural populations, the spread of the transgene into natural populations will depend on the relative mobility of the genetic construct, the magnitude of the negative fitness

effect of the genetic construct on the host, and the gene flow among host populations (GINZBURG *et al.* 1984; RIBEIRO and KIDWELL 1994). If Wolbachia-induced cytoplasmic incompatibility is used to drive transgenes into populations, then the initial frequency of the transformed hosts must exceed a threshold, and the threshold density will be determined by the maternal transmission rate of Wolbachia, the relative fitness of transformants, and the strategies used to introduce the transgenes (TURELLI and HOFFMANN 1999). Precise estimates of the effects of the resistance genes on the mosquito host will provide critical information toward developing strategies for successful transgene introduction and for evaluating their potential for establishment and spread in natural populations.

In conclusion, continued studies on the molecular basis and the physiology of resistance to the Plasmodium parasite are needed to facilitate our understanding of the mechanisms influencing fitness in resistant mosquito populations. This study suggests that the reduced fitness in our resistant population had a genome-wide effect on the genetic makeup of a mixed (resistant and susceptible) population. Constant exposure to parasites resulted in the maintenance of alleles linked with resistance in the population. However, the magnitude of the effects of individual loci determining resistance on mosquito fitness remains unknown. The relationship between fitness and genes determining resistance can also be better understood by direct comparison of reproductive success between isogenic mosquito lines or through QTL mapping of both the resistance and fitness traits simultaneously. Estimates of the cost of resistance can likely be obtained through direct fitness comparisons between transgenic-resistant and untransformed mosquitoes.

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