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COSTS AND BENEFITS OF MOSQUITO REFRACTORINESS TO MALARIA PARASITES: IMPLICATIONS FOR GENETIC VARIABILITY OF MOSQUITOES AND GENETIC CONTROL OF MALARIA

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Abstract.—The problem of fitness costs associated with host resistance to parasitism is related to the evolution of parasite virulence, population genetic diversity and the dynamics of host-parasite relationships, and proposed strategies for disease control through the genetic manipulation of mosquito vectors. Two *Aedes aegypti* populations, refractory and susceptible to *Plasmodium gallinaceum*, were previously selected from the Moyo-In-Dry strain (MOYO) through inbreeding ($F = 0.5$). Reproductive success and survivorship of the two populations were compared, and the influence of the parasite on mosquito fitness also was evaluated. Fitness components studied include fecundity, adult survivorship and egg-to-adult developmental time, blood-meal size, and adult body size. The refractory population has a significantly shorter egg-to-adult developmental time and a smaller body size, takes a smaller blood meal, and subsequently lays fewer eggs than the susceptible population. The mean longevity of the refractory population is significantly shorter than the susceptible population. Exposure to the parasite exhibited little effect on the survivorship and fecundity of either population. Several factors may contribute to the lower fitness of the refractory population, including founder effect, inbreeding depression, the effect of other uncharacterized genes linked to genes conferring refractoriness, and pleiotropic effects associated with these genes. The results are discussed in relation to the genetic diversity of natural mosquito populations and their implications for the genetic control of malaria.

Key words.—*Aedes aegypti*, coevolution, parasitism, *Plasmodium gallinaceum*, resistance, susceptibility, vector competence.

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The fitness trade-off between costs and benefits of resistance to parasitism or insecticides has recently received increased attention (McKenzie and Game 1987; Lenski 1988; Wakelin and Blackwell 1988; Turner 1990; Minkoff and Wilson 1992; Groeters et al. 1994). Such a fitness trade-off could influence the evolution of parasite virulence (May and Anderson 1983; Ewald 1994), the establishment and maintenance of sexual reproduction and male secondary sexual traits (Jaenike 1978; Bremermann 1980; Hamilton 1980; Hamilton and Zuk 1982; Tooby 1982), the population genetic diversity and dynamics of hosts and parasites (Haldane 1949; Hamilton 1982; Anderson 1988), and proposed strategies for disease control through the genetic manipulation of vectors (Collins and Besansky 1994). Parasites are intimately dependent upon their hosts for survival and often have deleterious effects on host reproductive success due to physical damage during their invasion and migration, release of toxins, and usurpation of host resources. Thus, parasite-imposed selection could lead a host population to complete resistance or to a point where additive genetic variance of susceptibility is depleted. Natural host populations exhibit considerable genetic variation in resistance to parasitism. Coevolutionary theory on host-parasite interactions suggests that the relationship between the negative fitness effects of parasites on hosts and fitness costs associated with resistance is in part responsible for the maintenance of genetic variation in host resistance (Haldane 1949; Leonard 1977; Leonard and Czocher 1980). This theory is supported by mathematical analyses (May and Anderson 1983) and experimental evidence from the interactions of bacteria and their viruses or plasmids (Levin and Lenski 1983).

In this study, we examined the fitness costs associated with resistance to the avian malaria parasite, *Plasmodium gallinaceum*, in its natural vector, *Aedes aegypti* mosquitoes. Following ingestion in a blood meal from an infected vertebrate host, the malaria parasite must complete a series of complicated biological events within the mosquito. Successful development includes exflagellation, fertilization, ookinete formation, and penetration through the peritrophic matrix and midgut epithelium to form oocysts. Oocysts then mature and develop into infective sporozoites, which then migrate to the salivary glands. Failure to complete any of these events inhibits parasite development and prevents transmission to the vertebrate host. Several parasite-inhibiting mechanisms have been recognized in mosquitoes. One mechanism involves the failure of the parasite to exflagellate or to undergo syngamy in the midgut of mosquitoes, probably because mosquitoes lack an exflagellation stimulator (Nijhout 1979). A second is the requirement for activation of parasite chitinase for penetration of the peritrophic matrix (Shahabuddin et al. 1993). A third is the melanotic encapsulation of the developing oocyst, which usually occurs extracellularly between the basolateral epithelial cell membrane and the basement membrane (Collins et al. 1986; Paskewitz et al. 1988). A fourth is salivary-gland barriers that prevent sporozoite penetration or reduce survivorship of sporozoites (Rosenberg 1985). A fifth is an apparent physiological incompatibility between the mosquito and the parasite, commonly referred to as a refractory condition (Kilama and Craig 1969; Thathy et al. 1994; Severson et al. 1995). Although intracellular ookinete killing by midgut epithelial cells has been observed in the *Anopheles gambiae/P. gallinaceum* system (Vernick et al.

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1995), the biochemical and physiological basis for mosquito refractoriness remains unknown.

Mosquito refractoriness to *Plasmodium* exhibits complex modes of inheritance (Kilama and Craig 1969; Thathy et al. 1994; Severson et al. 1995; Vernick et al. 1995). Quantitative trait locus (QTL) mapping, using restriction fragment length polymorphism (RFLP) markers, demonstrated that the susceptibility of *A. aegypti* to *P. gallinaceum* is determined by at least two unlinked loci, and that each locus exhibits a partial dominance effect on refractoriness (Severson et al. 1995). The contribution of each locus was found not to be equal. A locus identified on chromosome 2 has a major effect, while a locus on chromosome 3 a small effect. Ward (1963) observed a significant response to selection for reduced susceptibility, suggesting the heritability of this trait is fairly high. If the reproductive success of mosquitoes is reduced by parasite infection, even at a level that is undetectable in the laboratory, the selection pressure from the parasite could increase the frequency of refractory alleles within a population if there is no genetic trade-off between refractoriness and susceptibility. Therefore, one would expect a high frequency or fixation of refractory alleles in natural mosquito populations.

However, significant genetic variation in susceptibility to *P. gallinaceum* in natural and laboratory *A. aegypti* populations has been documented (Ward 1963; Kilama 1973; Thathy et al. 1994). In particular, these studies identified high levels of susceptibility among Asian and New World populations, and relative high levels of refractoriness in African populations. Rausher and Simms (1989) describe several mechanisms concerning plant/herbivore interactions that may apply to this phenomenon. First, genetic variation in host susceptibility may not be due to selection by the parasite, rather it may simply be a result of founder effects. Although several studies have demonstrated reduced fecundity in mosquitoes exposed to vertebrate hosts infected with malaria parasites (Hacker and Kilama 1974; Freier and Friedman 1976), it is unclear to what degree this effect can be attributed to the malaria parasite itself. That is, blood from an infected animal may be of reduced nutritive quality to the mosquito (Bennett 1970). Infected hosts also may be less attractive, so that fewer mosquitoes feed on infected hosts, and those that do imbibe a smaller blood meal (Freier and Friedman 1976). In addition, the prevalence of malaria parasites in natural mosquito populations, even in areas with high vertebrate disease transmission, is low (Paul et al. 1995). Genetic variability for refractoriness may be selectively neutral and not directly associated with fitness, but simply the result of genetic drift or other ecological constraints.

A second hypothesis is that genetic variation in susceptibility is subject to stabilizing selection, resulting in intermediate levels of refractoriness. That is, if infection by a parasite reduces host fitness, but refractoriness is costly for the host to produce and maintain, stabilizing selection for an intermediate level of refractoriness could occur. Fitness costs of refractoriness may include self-inflicted toxicity, disruption of previous functions for biological pathways that are involved in refractoriness, devotion of resources to resistance and away from other fitness-enhancing functions, or other pleiotropic effects of genes involved in refractoriness (Simms and Triplett 1994). For example, the melanotic encapsulation of parasites by mosquitoes requires tyrosine as a precursor, which also is important for other biological events, such as egg-chorion and cuticular tanning and wound healing (Li et al. 1994). Indeed, reduced ovary size and protein content, and delayed oviposition have been observed in mosquitoes actively encapsulating parasites (Ferdig et al. 1993). When refractoriness is associated with reduced fitness, a negative additive genetic correlation exists between refractoriness and mosquito fitness measured in a parasite-free environment. Therefore, this hypothesis predicts that the frequency of refractory alleles should be higher in endemic regions that in nonendemic areas, because selection pressure from the parasite would be stronger in endemic regions. In areas without the parasite, refractory alleles should be rare.

A third hypothesis is that the observed genetic variation in susceptibility is a transient state prior to complete refractoriness or susceptibility. If there are no negative fitness effects associated with refractoriness, parasite-imposed selection could ultimately result in fixation for refractory alleles. The time to fixation may be very long, because alleles for the refractory condition usually have dominant or partially dominant effects (Severson et al. 1995). On the other hand, when the parasite exerts no selection and the fitness costs of refractoriness are high, a polymorphic population could rapidly become completely susceptible.

The aims of the present study were: (1) to determine whether exposure to the malaria parasite, *P. gallinaceum*, affected fitness in the mosquito, *A. aegypti*; (2) to determine whether there were fitness costs associated with refractoriness; and (3) to determine whether refractory mosquitoes obtained a fitness advantage in the presence of the parasite. Fitness components examined included body size, blood-meal acquisition and size, female fecundity, larval-to-adult developmental time, time to initial oviposition, and adult survivorship. These traits may be interrelated, and may have important consequences for mosquito population dynamics and disease transmission. Fitness components affected by parasitism have the potential to evolve under parasite-imposed selection. The results are discussed in relation to genetic diversity of natural mosquito populations and to the genetic control of malaria.

Materials and Methods

Mosquitoes and Parasites.—Two populations of *A. aegypti* were used in this study. The MOYO-R and MOYO-S populations were selected for refractoriness to *P. gallinaceum*, and susceptibility from the Moyo-In-Dry strain (MOYO) by four generations of recurrent sib mating with a founder density of a pair of mosquitoes each (Thathy et al. 1994). Thus, the two populations have an inbreeding coefficient (F) of 0.5 (Crow and Kimura 1970). The MOYO strain was originally collected from Shauri Moyo Village, Mombasa, Kenya, in 1974.

Unless otherwise stated, mosquitoes were reared as previously described (Christensen and Sutherland 1984). Because the traits of interest could be sensitive to environmental parameters, the two populations were reared under identical conditions. The differences observed between them are therefore likely due to their genetic divergence or the interaction

between genotype and environment, but not due to the environment itself. In particular, we controlled water, food availability, and larval density during development to adults. Cotton pads soaked in 0.3 M sucrose solution were constantly available to adults and were changed every two days. All mosquitoes were maintained in an environmental chamber regulated at 26.5 ± 0.5°C and 80 ± 5% RH under a 16-h light: 8-h dark light cycle. Four- to five-day-old female mosquitoes were deprived of sucrose for 24 h before receiving an infected or uninfected blood meal.

Maintenance of the *P. gallinaceum* parasite was as previously described (Thathy et al. 1994). Female mosquitoes (50 mosquitoes/carton of 0.473 L), were allowed to engorge on restrained white leghorn chicks (Northern Hatchery Inc., Beaver Dam, WI). Parasite-infected chicks were obtained by sporozoite transmission. The parasitemia and percentage of gametocytes were monitored daily beginning one-week postinfection (PI). When the parasitemia (percentage of infected red blood cells) of an infected chick reached at least 10% with greater than 1% gametocyte development, the naive mosquitoes were allowed to bloodfeed on the infective chick for 15 min. Following exposure to chicks, fully engorged mosquitoes were separated and continuously supplied with sucrose pads. Mosquito susceptibility was examined by dissecting individual mosquitoes six to seven days post bloodfeeding, and scoring the number of oocysts that developed on the midgut.

Mosquito Body Size.—Wing length is a reliable measurement of adult mosquito size (e.g., Ward 1963; Kelly and Edman 1992). Adult size may influence survivorship, developmental time, and ability to acquire a blood meal. Wing length was measured from the axial incision to the apical margin, not including the fringe of scales (Kelly and Edman 1992). Two independent trials were conducted. For trial 1, we measured 50 male and 50 female mosquitoes of each population. Only females ($n = 50$ per population) were measured in trial 2.

Blood-Meal Size.—Blood-meal size influences not only mosquito fecundity (Woke et al. 1956), but also the potential for acquiring pathogens (Kershaw et al. 1955). Blood-meal size was estimated indirectly by measuring hemoglobin concentration ingested by a mosquito, using the HiCN method (Briegel et al. 1979) with slight modifications. The bloodfed females were dissected and the midgut containing blood from a single mosquito was placed into a test tube with 0.5 mL of Drabkin's solution (see Briegel et al. 1979). The mixture was ground thoroughly and incubated at room temperature for at least 30 min, then 400 μL of chloroform was added, and the mixture was vortexed and centrifuged at 1500 rpm for 5 min. The supernatant was transferred to a test tube with 800 μL distilled water, and vortexed briefly. The absorbance was read at 415 nm using a Hitachi model U-2000 spectrophotometer.

A calibration curve was prepared for each chick by using known amounts of chick blood (0.8, 1.6, 2.4, 3.2 and 4.0 μL) and measuring the corresponding absorbance. The absorbance of midguts taken from unfed females ($n = 10$) was also measured, and the average of their reading was subtracted from that of the bloodfed females. Two independent trials were conducted. In trial 1, 50 females of each population were exposed to the same uninfected or infected 17-day-old chick for 15 min. Twenty-five mosquitoes from each of the four treatments (2 populations × 2 infections = 4) were randomly selected to determine blood-meal size and wing length. In trial 2, 50 females from each population were exposed to an uninfected seven-day-old chick for 15 min. This experiment tests for a difference in blood-meal size between the MOYO-R and MOYO-S populations, effects of chick infection status and chick age on blood-meal size, and a correlation between mosquito body size and blood-meal volume.

Larval-to-Adult Developmental Time.—Sixty newly hatched larvae of each population were placed individually into glass vials (27.5 cm^3) containing 2 mL of double-distilled water. Small amounts of finely ground fish food (Tetramin) were added to each vial every two days. Vials were checked at three-hour intervals beginning on day 6, and the time period during which an adult mosquito emerged and its gender were recorded. Larvae that failed to develop into adults ($n = 13$ for MOYO-R and $n = 15$ for MOYO-S) were excluded from the analysis.

Mosquito Adult Survivorship.—The survivorship of males and females of the two populations was examined separately. Male and female pupae were collected from stock population, and placed into 0.473-L paper cartons (50 individuals each). After emergence, male and female mosquitoes of each population were placed in 10 cartons (50 individuals per cartons) with marquisette coverings. Sucrose pads were changed every two days. On day 5 postemergence, female mosquitoes were starved overnight and allowed to feed on uninfected or infected chicks, and then placed into cartons in the same manner as the males. Mosquitoes were examined daily, and dead individuals were counted and removed until all individuals had died. We started with 500 mosquitoes for each treatment and each population except the MOYO-R infected treatment ($n = 400$). At day 6–7 postinfection, 85–100 mosquitoes of each population were dissected to monitor *P. gallinaceum* infection.

Ovipositional Studies.—Fifty females of each population were allowed to mate with males (50/carton) for four days prior to bloodfeeding. Fully engorged females then were placed individually in glass vials, each containing a strip of filter paper (10 × 50 mm^2) as an oviposition substrate and about 1.5 mL of double-distilled water, and covered with marquisette. Sucrose was provided each day until day 5 post-bloodfeeding. Each female then was transferred to a fresh vial, and monitored for another five days. Female mosquitoes usually start to lay eggs two to four days after a blood meal. Excess water was discarded, and eggs were counted. The filter strips containing eggs were dried for one week, the eggs then hatched, and number of larvae was scored. Because both populations were exposed to the same infected or uninfected chick, any fecundity difference between the two populations within a treatment should not be influenced by differences in the blood-meal source. Parasite susceptibility was monitored by dissecting 16 females of each population at day 6 postinfection. An additional experiment was conducted to determine the time to initial oviposition. The basic protocol and design were as described above. Individual females were checked every three hours, beginning 36 hours after the blood

TABLE 1. ANOVA for the effects of mosquito populations and exposure to parasite on infection intensity and fitness components.

Experiment	Source of variation	df	Mean square	F	P
A. Mosquito susceptibility*	Population	1	139.923	15.89	< 0.0001
	Trial	1	0.662	0.08	0.78
	Population × trial	1	6.561	0.75	0.39
	Error	107	8.800		
B. Body size (females)†	Population	1	0.109	131.19	< 0.0001
	Trial	1	0.002	1.81	0.18
	Population × trial	1	0.005	5.68	0.02
	Error	188	0.001		
Body size (males)	Population	1	0.004	4.24	0.04
	Error	94	0.001		
C. Blood-meal size‡	Population	1	160.482	37.27	< 0.0001
	Chick infection status	1	18.129	4.21	0.04
	Population × chick infection status	1	8.678	2.02	0.16
	Error	145	4.306		
D. Larva-to-adult developmental times§	Population	1	0.163	40.98	< 0.0001
	Sex	1	0.081	20.36	< 0.0001
	Population × sex	1	0.005	1.26	0.27
	Error	88	0.004		
E. Ovipositional studies					
Fecundity¶	Population	1	228.035	0.71	0.40
	Exposure to parasite	1	13444.732	41.74	< 0.0001
	Population × exposure to parasite	1	1120.212	3.48	0.06
	Error	179	322.110		
Hatchability#	Population	1	2.192	33.05	< 0.0001
	Exposure to parasite	1	0.015	0.23	0.63
	Population × exposure to parasite	1	0.116	1.75	0.19
	Error	145	0.066		
Time to initial oviposition††	Population	1	0.214	20.31	< 0.0001
	Exposure to parasite	1	0.038	3.62	0.06
	Population × exposure to parasite	1	0.025	2.41	0.12
	Error	161			

Note: MS of error was used as the denominator in all F-tests.
* Dependent variable is infection intensity. Data are square-root transformed.
† Dependent variable is wing length. Data are *ln* transformed for males and females.
§ Dependent variable is larva-to-adult developmental time. Data are *ln* transformed.
¶ Dependent variable is number of eggs laid. Data are not transformed.
# Dependent variable is proportion of eggs hatched. Data are arcsine transformed.
†† Dependent variable is the time to initial oviposition. Data are *ln* transformed.

meal, and the time period required for the first eggs to be deposited was recorded.

Data Analysis.—We tested each dataset for normality using the Shapiro-Wilk test (SAS Institute1994) and for homogeneity using the F-max test (Sokal and Rohlf 1981). The data were transformed when appropriate (Table 1). Analysis of variance (ANOVA) then was used to test for the effect of population on body size and larvae-to-adult developmental time, and the effect of population and exposure to infected hosts on blood-meal size, fecundity, and time to initial oviposition. When appropriate, orthogonal contrasts were used to compare the populations and/or infection treatments with controls. Survivorship data were analyzed using the Kalpan-Meier method (SAS Institute 1994), and the log-rank test results are presented.

TABLE 2. Susceptibility of two *Aedes aegypti* populations.

Experiment	Population	No. of mosquitoes examined	Mean infection intensity ± SD (range)	Prevalence (%)
Oviposition	MOYO-R*	16	1.5 ± 0.7 (1–2)	12.5
	MOYO-S†	16	54.6 ± 33.5 (13–137)	87.5
Survivorship	MOYO-R	85	4.4 ± 3.9 (1-11)	14.1
	MOYO-S	99	43.0 ± 44.9 (1–270)	83.8

* MOYO-R refers to the proportion refractory to the malaria parasite, *Plasmodium gallinaceum*, selected from MOYO strain (see text for details).
† MOYO-S refers to the population susceptible to *P. gallinaceum*, also selected from MOYO.

RESULTS

Mosquito Susceptibility

The mean infection intensity (the number of malaria oocysts in an infected mosquito) and prevalence (the proportion of mosquitoes infected in a population) of the mosquitoes used in the ovipositional and survivorship experiments are presented in Table 2. The two independent trials yielded similar infection levels. The MOYO-S population was significantly more susceptible than MOYO-R (Table 1). The majority of MOYO-R mosquitoes harbored no parasites, and none of these mosquitoes had more than 11 oocysts. Overall, 84.3% of the MOYO-S females were susceptible, and 73.4% of these infected individuals had more than 11 oocysts (oo-

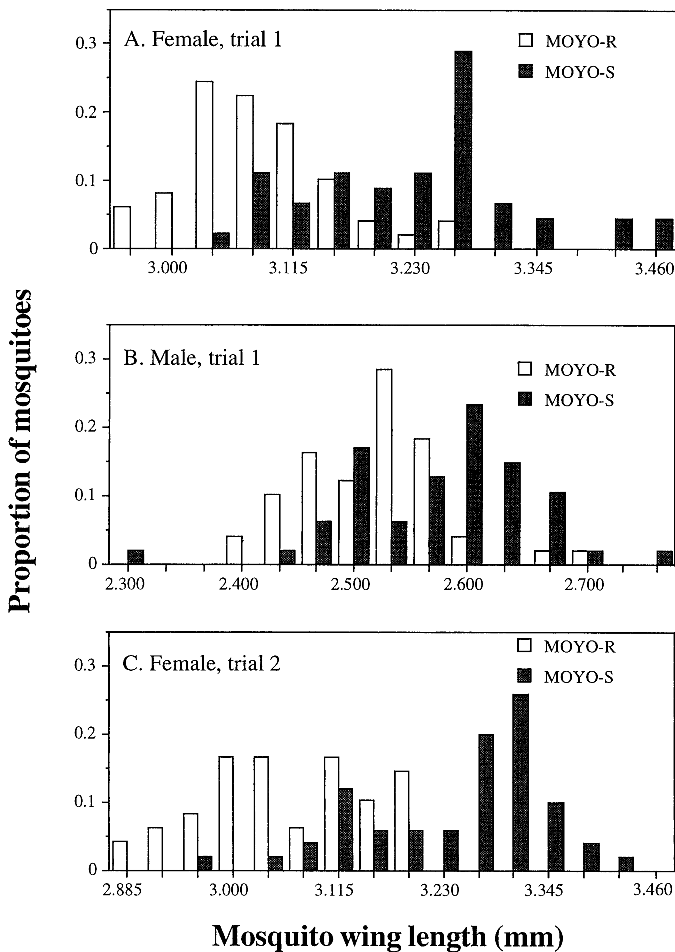

FIG. 1. Distribution of wing lengths in two *Aedes aegypti* populations, susceptible (MOYO-S) and refractory (MOYO-S) to *Plasmodium gallinaceum*. Two independent trials were conducted. Only female wing length was measured in trial 2.

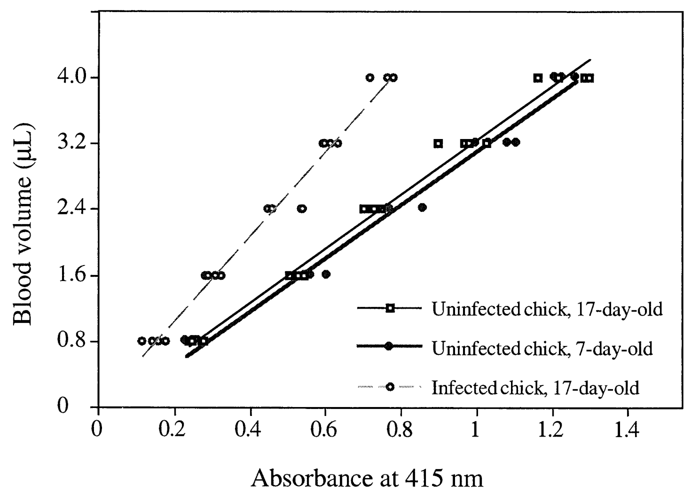

FIG. 2. Calibration curves for estimating blood-meal size, using HiCN method (see text for detail). Linear regression was used to describe the relationship between the known amount of blood (Y) and spectrophotometrical reading (X). Y = −0.045 + 3.281 X, r^2 = 0.988, df = 19, $P < 0.0001$ for uninfected, 17-day-old chick. Y = −0.104 + 3.181 X, r^2 = 0.978, df = 19, $P < 0.0001$ for uninfected, 7-day-old chick. Y = −0.065 + 5.007 X, r^2 = 0.979, df = 19, $P < 0.0001$ for infected, 17-day-old chick.

cyst distribution not shown). Our infection data for the two populations were similar to those reported by Thathy et al. (1994).

Mosquito Body Size

Since mosquitoes exhibit sexual dimorphism in body size, and the data combining both sexes did not meet the assumptions of normality and homogeneity required for analysis of variance (ANOVA), we analyzed the male and female body size data separately. MOYO-R females were significantly smaller than MOYO-S females, as revealed by wing length (Fig. 1; trial 1: 3.11 vs. 3.22 mm, ANOVA orthogonal contrast, $t = 6.41$, $P < 0.0001$; trial 2: 3.06 vs. 3.24 mm, $t = 9.87$, $P < 0.0001$). Similar results were found in males (2.54 mm for MOYO-R vs. 2.57 mm for MOYO-S, $P = 0.04$). There was no significant difference in female wing length between the two trials (Table 1).

Blood-meal Size

Linear regression was used to establish correlations between absorbance at 415 nm and actual blood volume for the thre chicks used in this experiment (Fig. 2). The regression coefficient for the 17-day-old uninfected chick was not significantly different from the seven-day-old uninfected chicks (ANOVA, $F = 2.36$; df = 1, 14; $P > 0.05$), however, it was significantly different from the 17-day-old infected chick ($F = 17.57$; df = 1, 14; $P < 0.001$). This is due to a reduced hemoglobin content in blood from infected chicks (Fig. 2; Bennett 1970).

There was no significant difference in blood-meal size within treatments between the two independent trials (Table 3; ANOVA, $F = 3.87$; df = 1, 96; $P > 0.05$), therefore the data for the two trials were combined for subsequent analysis. The proportion of MOYO-R females that took a blood-meal was smaller, and the average blood-meal size for MOYO-R was significantly less than for MOYO-S when they were exposed to either infected or uninfected chicks (Tables 1, 3, ANOVA orthogonal contrasts, $t = 4.09$, df = 1, $P < 0.0001$ for uninfected chicks; $t = 4.56$, df = 1, $P < 0.0001$ for infected chicks). Since the chick was restrained while feeding, defensive behaviors by the animal likely were not re-

TABLE 3. Blood-meal size differences between two *Aedes aegypti* substrains refractory and susceptible to *Plasmodium gallinaceum*.

Infection status and age of chicks	Substrain	No. of mosquitoes examined	Mean blood-meal size ± SD (µL)	Range (µL)	% of mosquitoes taking any blood
Infected,	MOYO-R	24	0.25 ± 0.85	0–3.03	13
17-day-old	MOYO-S	25	2.97 ± 2.91	0–8.43	64
Uninfected,	MOYO-R	25	1.30 ± 1.75	0–4.96	52
17-day-old	MOYO-S	25	2.62 ± 2.35	0–8.16	84
Uninfected,	MOYO-R	25	1.71 ± 1.81	0–5.82	60
7-day-old	MOYO-S	25	3.79 ± 2.08	0–6.95	88

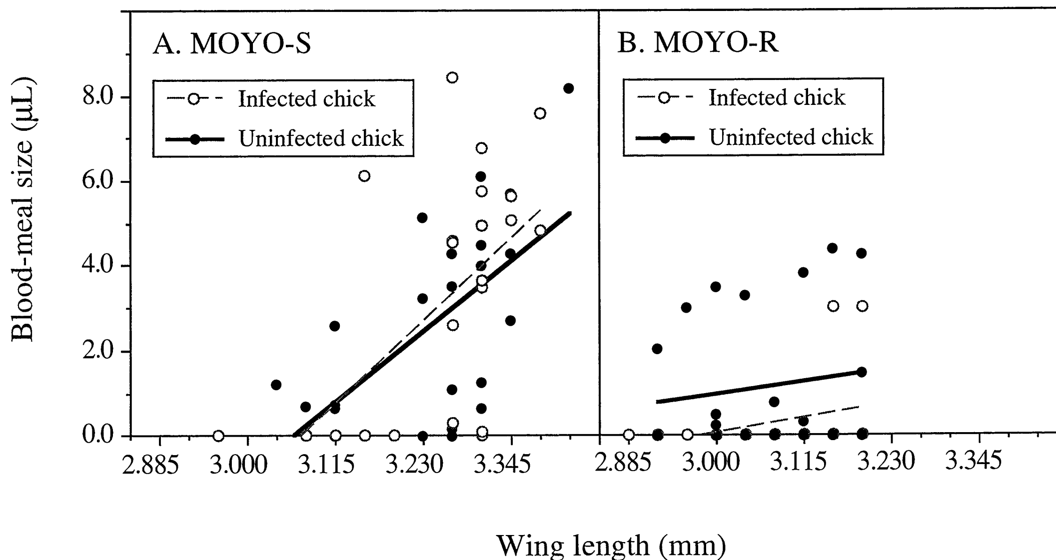

FIG. 3. Correlations between mosquito body size and blood-meal size. Each mosquito population was exposed to an infected and a uninfected chick. Unfed individuals were included in correlation analysis. For MOYO-S, r^2 = 0.39, df = 24, $P < 0.001$ when exposed to the uninfected; r^2 = 0.35, df = 24, $P < 0.01$ when exposed to the infected chick. For MOYO-R, r^2 = 0.02, df = 24, $P > 0.05$ when exposed to the uninfected chick; r^2 = 0.13, df = 23, $P > 0.05$ when exposed to the infected chick.

sponsible for the difference in blood-meal size between the two populations. When exposed to the infected chick, MOYO-R females ingested less blood as compared to those exposed to uninfected chicks (Table 1; t = 2.44, df = 1, $P <$ 0.05), but no significant difference was found in MOYO-S (t = 0.45, df = 1, $P > 0.05$).

We further examined whether mosquito body size was, in part, responsible for the observed variations among individuals within a population or between populations. Simple correlation coefficients between wing length and blood-meal size were calculated for the two populations exposed to either infected or uninfected chicks. When all individuals were included in the analysis, significant correlations were found in MOYO-S population (Fig. 3A), but not in MOYO-R (Fig. 3B). When individuals not bloodfed were excluded, only one correlation was significant, that is, MOYO-S exposed to uninfected chicks (r^2 = 0.38, df = 20, $P < 0.01$).

Larva-to-Adult Developmental Time

The MOYO-R population had a significantly shorter larva-to-adult developmental time than did MOYO-S for both males (Tables 1, 4; ANOVA orthogonal contrast, t = 3.66, df = 1, $P < 0.001$) and females (t = 5.43, df = 1, $P <$ 0.0001). The faster development of MOYO-R may be related to its smaller body size.

TABLE 4. The larva-to-adult developmental time for two *Aedes aegypti* populations.

Population	Sex	*N*	Mean larval-to-adult developmental time ± SD (h)	Range (h)
MOYO-R	Female	25	186.98 ± 13.08	172–222
	Male	22	178.75 ± 11.68	167–214
MOYO-S	Female	23	206.24 ± 10.43	186.5–222
	Male	22	191.68 ± 12.98	172–207.5

Adult Mosquito Survivorship

Survivorship curves of MOYO-R and MOYO-S are illustrated in Figure 4. MOYO-R male mosquitoes had a significantly lower survivorship than did MOYO-S males (Fig. 4A; χ^2 = 64.88, df = 1, $P < 0.0001$). The 50% survival point was day 20–21 postemergence for MOYO-R males (mean longevity = 20.06 d) and day 23–24 postemergence for MOYO-S males (mean longevity = 24.27 d). Compared to MOYO-S females, the survivorship of MOYO-R females was reduced dramatically (Fig. 4B). The 50% survivorship occurred at day 26–27 postexposure for MOYO-R females and at day 47–48 for MOYO-S females when uninfected, or at day 26 for MOYO-R females and at day 49–50 for MOYO-S females when infected. The mean longevity of MOYO-R females was nearly half that of MOYO-S females, whether they were uninfected (25.11 vs. 45.67 d; χ^2 = 429.19, df = 1, $P < 0.0001$) or infected (26.69 vs. 45.06 d; χ^2 = 325.57, df = 1, $P < 0.0001$). Exposure to *P. gallinaceum* had no detectable effect on female survivorship for either population (Fig. 4B: χ^2 = 1.40, df = 1, $P > 0.05$ for MOYO-R; χ^2 = 0.01, df = 1, $P > 0.05$ for MOYO-S).

Ovipositional Studies

MOYO-R females produced a similar number of eggs as MOYO-S females when they were exposed to an infected chick (Table 5; ANOVA orthogonal contrast, t = 0.72, df = 1, $P > 0.05$). When they were exposed to an uninfected chick, more eggs were laid by MOYO-S than MOYO-R, and the difference was marginally significant (Tables 1, 5; t = 1.92, df = 1, P = 0.056). MOYO-R mosquitoes exposed to *P. gallinaceum* laid 14.3% fewer eggs than those exposed to an uninfected blood meal (Tables 1, 5; t = 3.16, $P < 0.01$), while the reduction was 23.9% for MOYO-S (t = 6.07, df = 1, $P < 0.0001$). Because MOYO-R population is refractory to *P. gallinaceum*, the reduced fecundity in this population,

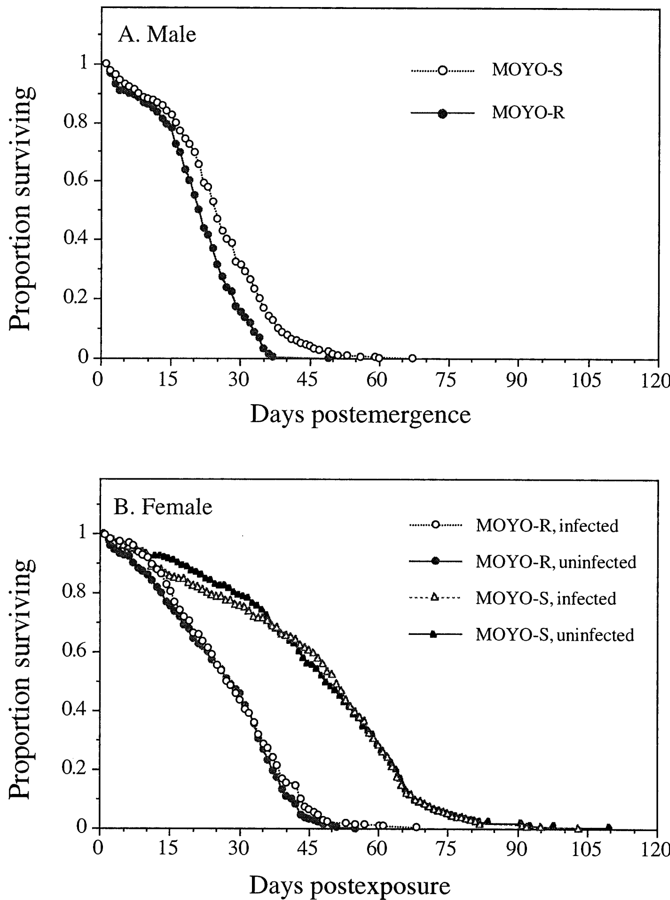

FIG. 4. Survivorship curves for MOYO-R and MOYO-S. Females of each population were exposed to *Plasmodium gallinaceum* infected or uninfected chicks.

exposed to an infected chick, may reveal infected vertebrate host effects, for example, degraded blood quality. The parasite itself may contribute directly to the greater reduction in fecundity of *P. gallinaceum*–infected MOYO-S females.

Infection status of the blood meal did not affect egg hatchability in either population (Tables 1, 5; ANOVA orthogonal contrast, $t = 1.55$, df = 1, $P > 0.05$ for MOYO-R; $t = 0.52$, df = 1, $P > 0.05$ for MOYO-S). However, a significant difference between the two populations was observed. A greater proportion of MOYO-S eggs hatched as compared to MOYO-R eggs when the mosquitoes received either an uninfected (83% vs. 57%, $t = 3.91$, df = 1, $P < 0.001$) or an infected blood meal (81% vs. 64%, $t = 4.23$, df = 1, $P < 0.0001$).

The time to initial oviposition was significantly delayed in MOYO-R females as compared to the MOYO-S females, when they each received an uninfected blood meal (Tables 1, 6; ANOVA orthogonal contrast, $t = 2.04$, df = 1, $P < 0.05$) or an infected blood meal ($t = 4.39$, df = 1, $P < 0.0001$). This trait was not affected by the infection status of the blood meal with MOYO-S mosquitoes ($t = 0.26$, df = 1, $P > 0.05$). However, the delay in oviposition in MOYO-R females was significantly greater when they received an infected as compared to an uninfected blood meal ($t = 2.34$, df = 1, $P < 0.05$).

TABLE 6. Mean time to the initiation of oviposition for two *Aedes aegypti* populations.

Population	Mosquito infection status	No. of mosquitoes examined	Mean time to oviposition (h) ± SD	Range (h)
MOYO-R	Control	35	55.80 ± 7.32	51–81
	Infected	40	59.25 ± 9.67	51–81
MOYO-S	Control	44	52.91 ± 2.83	51–60
	Infected	46	53.28 ± 4.39	51–78

DISCUSSION

Our results demonstrate a reduction in fitness in an *A. aegypti* population (MOYO-R) that is refractory to *P. gallinaceum* under our experimental conditions, when compared to a susceptible population (MOYO-S) (Table 7). The decreased fitness of MOYO-R was manifested in several fitness components. MOYO-R has a significantly shorter egg-to-adult developmental time and a smaller adult body size, took a smaller blood meal, and subsequently laid fewer eggs than MOYO-S. The mean longevity of MOYO-R was significantly shorter than that of MOYO-S, regardless of the infection status of the blood meal. Exposure to the parasite had little effect on fecundity and no detectable effect on the survivorship of either population. Because the two mosquito populations were derived from the same ancestor population, and because all experiments were conducted in the same controlled environment, the observed fitness differences between the two mosquito populations most likely resulted from their genetic divergence.

If mosquito refractoriness to *Plasmodium* is associated with fitness costs, the frequency of alleles conferring refractoriness should decrease in polymorphic populations in the absence of selective pressure by the parasite. The rate of decrease may be a function of the initial frequency of refractory alleles, the fitness of homozygotes and heterozygotes, and the mating structure among the genotypes. The pattern of allele frequency change over generations may be complex because of

TABLE 5. The effects of exposure to the parasite and *Aedes aegypti* substrain on fecundity and hatchability.

Substrain	Mosquito infection status	No. of mosquitoes examined	Mean number of eggs laid ± SD (range)	Mean proportion of eggs hatched ± SD (range)
MOYO-R	Control	44	85.41 ± 15.92 (34–112)	0.57 ± 0.23 (0.01–0.87)
	Infected	42	73.19 ± 14.38 (44–94)	0.64 ± 0.20 (0.00–0.98)
MOYO-S	Control	48	92.60 ± 20.66 (35–133)	0.83 ± 0.22 (0.22–0.99)
	Infected	49	70.47 ± 19.47 (10–102)	0.81 ± 0.19 (0.00–1.00)

TABLE 7. Summary of differences in susceptibility and fitness components between two *Aedes aegypti* populations refractory (MOYO-R) and susceptible (MOYO-S) to *Plasmodium gallinaceum.*

Traits analyzed	Comparison between MOYO-R and MOYO-S
A. Susceptibility to *P. gallinaceum*	MOYO-S > MOYO-R
B. Adult body size	
females	MOYO-S > MOYO-R
males	MOYO-S > MOYO-R
C. Blood-meal size	
when exposed to an uninfected vertebrate host	MOYO-S > MOYO-R
when exposed to an infected vertebrate host	MOYO-S > MOYO-R
D. Larva-to-adult developmental time	
females	MOYO-S > MOYO-R
males	MOYO-S > MOYO-R
E. Fecundity	
uninfected mosquitoes	MOYO-S > MOYO-R
infected mosquitoes	MOYO-S = MOYO-R
F. Hatchability	MOYO-S > MOYO-R
G. Time to initial oviposition	
uninfected mosquitoes	MOYO-S < MOYO-R
infected mosquitoes	MOYO-S < MOYO-R
H. Mosquito survivorship	
males	MOYO-S > MOYO-R
uninfected females	MOYO-S > MOYO-R
infected females	MOYO-S > MOYO-R

the multigenic nature of refractoriness (Severson et al. 1995), and there is some experimental evidence to support this prediction (Kilama 1973). That is, significant decreases in refractoriness were observed over about eight generations in several artificial populations that were initiated with varying proportions of susceptible and refractory genotypes. Only some minor allelic frequency fluctuations were observed in populations fixed for the susceptible or refractory genotypes over the same time period (Kilama 1973).

Our data suggest that *P. gallinaceum* likely imparts weak selection pressure on the mosquito host. The parasite exhibits no detectable effect on mosquito survivorship (Fig. 4), and little effect on fecundity (Table 5). Therefore, these results support the suggestion that pathogens or parasites transmitted by biting arthropod vectors should exhibit low virulence in the motile vector host (Ewald 1983). Successful parasite transmission depends on vector susceptibility to parasites, vector longevity, vigorous biting behavior, and other factors. Severely affected vectors would reduce parasite transmission. Our data also support the hypothesis that genetic variation in mosquito susceptibility is subject to selection for increased frequency of susceptible genes (see hypothesis 3 in the introduction). Therefore, we would predict a high frequency or even fixation of susceptible genes in natural mosquito populations. This is true for Asian and New World *A. aegypti* populations, but not for some African populations (Ward 1963; Kilama 1973). What causes such a paradox?

Careful examination of our data may provide insight as to why refractory alleles are maintained in certain African mosquito populations. The significantly shorter developmental time found in MOYO-R (Table 4), probably correlated with a smaller body size (Fig. 1), may provide the mosquitoes with a competitive advantage in environments where hosts, water availability, or other resources are limited. In such environments, early adult emergence may represent an important fitness-enhancing strategy. Although the refractory genotype exhibits a reduced fitness when exposed to our laboratory conditions, it may not be so in a harsh natural environment. For example, in some regions of Africa, particularly regions near the Sahara Desert where the rainy season is very short and water is scarce, mosquito eggs must hatch and develop into adults during a short window of opportunity. Such environmental conditions would favor any characters that reduce mosquito developmental time. If parasite refractory alleles originated in and were maintained by such ecological constraints, genetic correlations between refractoriness, body size, and developmental time are required. Although our data demonstrate phenotypic correlations between these traits, genetic correlations remain to be determined. Therefore, although we did not observe fitness trade-offs in mosquitoes between costs and benefits of refractoriness to the malaria parasite under our laboratory conditions, such fitness trade-offs may exist in some natural environments. Theories about host-parasite coevolution should consider the interactions between environments and host or parasite genotypes. In addition, the contributions of founder effects to the genetic diversity of natural mosquito populations need to be evaluated.

Our results may have implications for the genetic control of malaria. Malaria, one of the most important parasitic diseases today, infects 270 million people and results in 1–2 million deaths per year, primarily in children from tropical Africa (Knudsen and Slooff 1992). The emergence of pesticide resistance in mosquitoes and antimalaria drug resistance in *Plasmodium* has significantly limited malaria control efforts. Also, efforts toward vaccine development show little promise for disease control in the near future. Novel control strategies based on genetic disruption of mosquito vector competence have been proposed recently (James 1992; Collins and Besansky 1994). However, the success of genetic control strategies critically depends on the introduction and spread of parasite-inhibiting genes in natural vector populations. Understanding the fitness effects of these genes will provide important information toward developing such strategies. Two mechanisms have been proposed for driving parasite-inhibiting genes into mosquito populations (Curtis 1994). The first is for mosquitoes carrying refractory alleles to possess a competitive advantage, particularly in the presence of the parasite. The second is that refractory alleles may be spread by some driving force, such as mobile DNA sequences (e.g., transposons) or rickettsial symbionts (e.g., *Wolbachia*). If refractoriness is associated with high fitness costs and the parasite causes little damage, refractory alleles will not spread throughout the mosquito population without some driving mechanism. When such driving forces are used, the ultimate fate of refractory alleles that negatively influence host fitness will be determined by numerous factors. Investigation of these possibilities requires extensive theoretical (Ribeiro and Kidwell 1994) and experimental studies. The success of genetic control of malaria therefore requires an understanding of the genetic structure of natural mosquito populations.

Finally, the biochemical or genetic mechanisms underlying the observed fitness differences between MOYO-R and MOYO-S populations remain unknown. Three hypotheses, though not mutually exclusive, could explain the reduced fitness of MOYO-R. First, fitness variation between the two populations may reflect founder effect, inbreeding depression, or both. Since both populations were derived from the original MOYO population through selective inbreeding with a pair of mosquitoes as founders, it is possible that genes responsible for reduced fitness cosegregated with refractory genes. Although the effects of inbreeding are unknown for mosquitoes, genetic variation in inbreeding depression among lineages has been demonstrated in other insects (e.g., Pray and Goodnight 1995). RFLP data for several *A. aegypti* strains or crosses suggest that they have greater heterozygosity than expected (Severson et al. 1993; Severson and Kassner 1995), suggesting that mosquitoes may possess unknown mechanisms to avoid inbreeding. Second, the reduced fitness of MOYO-R may be caused by uncharacterized genes tightly linked to genes determining refractoriness. Third, genes conferring refractoriness may have direct pleiotropic effects on other factors determining fitness. Our recent population studies seem to refute the first hypothesis. By mixing MOYO-R with another highly susceptible strain (RED) and examining gene frequency changes over generations, we found that frequencies of the major QTL marker alleles specific to MOYO-R were decreased substantially after six generations (G. Yan, unpubl. data), but the frequencies of MOYO-R specific genes on chromosome 1 did not exhibit such changes. Similar results were observed for another independently selected refractory population (Kilama 1973): when the refractory population was mixed with a susceptible population in equal proportion, refractory gene (*pls*) frequency was reduced significantly in the first few generations. We have developed a linkage map for *A. aegypti* using RFLP arkers and have used these markers to identify two unlinked QTL affecting *P. gallinaceum* susceptibility (Severson et al. 1993, 1995). The genetic relationships between refractoriness to *Plasmodium* and traits associated with fitness can be identified using QTL mapping techniques (see Severson 1994). Such studies would add significantly to our understanding of the relationship between refractoriness and fitness.

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Literature Cited

Anderson, R. M. 1988. The population biology and genetics of resistance to infection. Pp. 233–263 *in* D. Wakelin and J. M. Blackwell, eds. Genetics of resistance to bacterial and parasitic infection. Taylor and Francis, London.

Bennett, G. F. 1970. The influence of the blood meal type on fecundity of *Aedes* (*Stegomyia*) *aegypti* L. (Diptera: Culicidae). Can. J. Zool. 48:539–543.

Bremermann, H. J. 1980. Sex and polymorphism and strategies in host-pathogen interactions. Journal of Theoretical Biology 87: 671–702.

Briegel, H., A. O. Lea, and M. J. Klowden. 1979. Hemoglobinometry as a method for measuring blood meal sizes of mosquitoes. J. Med. Entomol. 15:235–238.

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

Collins, F. H., and N. J. Besansky. 1994. Vector biology and the control of malaria in Africa. Science 264:1874–1875,

Collins, F. H., R. K. Sakai, K. D. Vernick, S. M. Paskewitz, D. C. Seeley, L. H. Miller, W. E. Collins, C. C. Campbell, and R. W. Gwadz. 1986. Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. Science 234:607–610.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper and Row, New York.

Curtis, C. F. 1994. The case for malaria control by genetic manipulation of its vector. Parasitol. Today 10:371–374.

Ewald, P. W. 1983. Host-parasite relations, vectors, and the evolution of disease severity. Annu. Rev. Ecol. Syst. 14:465–485.

———. 1994. Evolution of infectious disease. Oxford Univ. Press, Oxford.

Ferdig, M. T., B. T. Beerntsen, F. J. Spray, J. Li., and B. M. Christensen. 1993. Reproductive costs associated with resistance in a mosquito-filarial worm system. Am. J. Trop. Med. Hyg. 49:756–762.

Freier, J. E., and S. Friedman. 1976. Effect of host infection with *Plasmodium gallinaceum* on the reproductive capacity of *Aedes aegypti*. J. Invertebr. Pathol. 28:161–166.

Groeters, F. R., B. E. Tabashnik, and N. Finson. 1994. Fitness costs of resistance to *Bacillus thuringiensis* in the diamondback moth (*Plutella xylostella*). Evolution 48:197–201.

Hacker, C. S., and W. L. Kilama. 1974. The relationship between *Plasmodium gallinaceum* density and the fecundity of *Aedes aegypti*. J. Invertebr. Pathol. 23:101–105.

Haldane, J. B. S. 1949. Disease and evolution. La Ricerca Scient. Suppl. 19:68–76.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. Oikos 35:282–290.

———. 1982. Pathogens as causes of genetic diversity in their host populations. Pp. 269–296 *in* R. M. Anderson and R. M. May, eds. Population biology of infectious diseases. Springer, New York.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: A role for parasites? Science 218:384–387.

Jaenike, J. 1978. An hypothesis to account for the maintenance of sex within populations. Evol. Theory 3:191–194.

James, A. A. 1992. Mosquito molecular genetics: The hands that feed bite back. Science 257:37–38.

Kelly, R., and J. D. Edman. 1992. Mosquito size and multiple transmission of avian malaria in the laboratory. J. Am. Mosq. Control Assoc. 8:386–388.

Kershaw, W. E., M. M. J. Lavoipierre, and W. N. Beesley. 1955. Studies on the intake of microfilariae by their insect vectors, their survival, and their effect on the survival of their vectors. VII. Further observations on the intake of the microfilariae of *Dirofilaria immitis* by *Aedes aegypti* in laboratory conditions: The pattern of the intake of a group of flies. Ann. Trop. Med. Parasitol. 49:203–211.

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. 1969. Monofactorial inheritance of susceptibility to *Plasmodium gallinaceum* in *Aedes aegypti*. Ann. Trop. Med. Parasitol. 63:419–432.

Knudsen, A. B., and R. Slooff. 1992. Vector-borne disease problems in rapid urbanization: New approaches to vector control. Bull. WHO 70:1–6.

Lenski, R. E. 1988. Experimental studies of pleiotropy and epistasis in *Escherichia coli*. Variation in competitive fitness among mutants resistant to virus T4. Evolution 42:425–432.

LEONARD, K. J. 1977. Selection pressures and plant pathogens. Ann. N.Y. Acad. Sci. 287:207–222.

LEONARD, K. J., AND R. J. CZOCHER. 1980. Theory of genetic interactions among populations of plants and their pathogens. Annu. Rev. Phytopathol. 18:237–258.

LEVIN, B. R., AND R. E. LENSKI. 1983. Coevolution in bacteria and their viruses and plasmids. Pp. 99–127 *in* D. J. Futuyma and M. Slatkin, eds. Coevolution. Sinauer, Sunderland, MA.

LI, J., X. ZHAO, AND B. M. CHRISTENSEN. 1994. Dopachrome conversion activity in *Aedes aegypti*: Significance during melanotic encapsulation of parasites and cuticular tanning. Insect Biochem. Mol. Biol. 24:1043–1049.

MAY, R. M., AND R. M. ANDERSON. 1983. Epidemiology and genetics in the coevolution of parasites and hosts. Proc. R. Soc. Lond. B, Biol. Sci. 219:281–313.

MCKENZIE, J. A., AND A. Y. GAME. 1987. Diazinon resistance in *Lucilia cuprina*: Mapping of a fitness modifier. Heredity 59:371–381.

MINKOFF, C., AND T. G. WILSON. 1992. The competitive ability and fitness components of the methoprene-tolerant (met) *Drosophila* mutant resistant to juvenile hormone analog insecticides. Genetics 131:91–97.

NIJHOUT, M. M. 1979. *Plasmodium gallinaceum*: Exflagellation stimulated by a mosquito factor. J. Parasitol. 48:75–80.

PASKEWITZ, S. M., M. R. BROWN, A. O. LEA, AND F. H. COLLINS. 1988. Ultrastructure of the encapsulation of *Plasmodium cynomolgi* (B Strain) on the midgut of a refractory strain of *Anopheles gambiae*. J. Parasitol. 74:432–439.

PAUL, R. E. L., M. J. PACKER, M. WALMSLEY, M. LAGOG, L. C. RANFORD-CARTWRIGHT, R. PARU, AND K. P. DAY. 1995. Mating patterns in malaria parasite populations of Papua New Guinea. Science 269:1709–1711.

PRAY, L. A., AND C. J. GOODNIGHT. 1995. Genetic variation in inbreeding depression in the red flour beetle *Tribolium castaneum*. Evolution 49:176–188.

RAUSHER, M. D., AND E. L. SIMMS. 1989. The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect stabilizing selection. Evolution 43:563–572.

RIBEIRO, J. M. C., AND M. G. KIDWELL. 1994. Transposable elements as population drive mechanisms: Specification of critical parameters. J. Med. Entomol. 31:10–16.

ROSENBERG, R. 1985. Inability of *Plasmodium knowlesi* sporozoites to invade *Anopheles freeborni* salivary glands. Am. J. Trop. Med. Hyg. 34:687–691.

SAS INSTITUTE. 1994. JMP® user's guide. Cary, NC.

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

SEVERSON, D. W., AND V. A. KASSNER. 1995. Analysis of mosquito genome structure using graphical genotyping. Insect Mol. Biol. 4:279–286.

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., V. THATHY, A. MORI, Y. ZHANG, AND B. M. CHRISTENSEN. 1995. Restriction fragment length polymorphism mapping of quantitative trait loci for malaria parasite susceptibility in the mosquito *Aedes aegypti*. Genetics 139:1711–1717.

SHAHABUDDIN, M., T. TOYOSHIMA, M. AIKAWA, AND D. C. KASLOW. 1993. Transmission-blocking activity of a chitinase inhibitor and activation of malarial parasite chitinase by mosquito protease. PNAS USA 90:4266–4270.

SIMMS, E. L., AND J. TRIPLETT. 1994. Costs and benefits of plants responses to disease: Resistance and tolerance. Evolution 48: 1973–1985.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. Freeman, San Francisco, CA.

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.

TOOBY, J. 1982. Pathogens, polymorphism, and the evolution of sex. J. Theor. Biol. 97:557–576.

TURNER, S. J. 1990. The identification and fitness of virulent potato cyst-nematode populations (*Globodera pallida*) selected on resistant *Solanum vernei* hybrids for up to eleven generations. Ann. Appl. Biol. 117:385–397.

VERNICK, K. D., H. FUJIOKA, D. C. SEELEY, B. TANDLER, M. AIKAWA, AND L. H. MILLER. 1995. *Plasmodium gallinaceum*: A refractory mechanism of ookinete killing in the mosquito, *Anopheles gambiae*. Exp. Parasitol. 80:583–595.

WAKELIN, D., AND J. M. BLACKWELL. 1988. Genetics of resistance to bacterial and parasitic infection. Taylor and Francis, London.

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

WOKE, P. A., M. S. ALLY, AND C. R. ROSENBERGER. 1956. The number of eggs developed related to the quantities of human blood ingested in *Aedes aegypti* (L.). Ann. Entomol. Soc. Am. 49:435–441.

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