Lambornella clarki (Ciliophora: Tetrahymenidae) Resistance in *Aedes sierrensis* (Diptera: Culicidae): Population Differentiation in a Quantitative Trait and Allozyme Loci

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ABSTRACT Heritable variation for resistance to the parasite *Lambornella clarki* (Corliss & Coats) by its host *Aedes sierrensis* (Ludlow) and neutral genetic variation among local Oregon mosquito populations were assessed by selection and allozyme frequencies. *L. clarki* resistance was significantly greater in a population of mosquitoes normally exposed to this parasite than in an unexposed population. Despite this difference between populations, there was no significant increase in heritable resistance to *L. clarki* within populations of *Ae. sierrensis* after 1 generation of laboratory selection. Differentiation estimated from allozyme electrophoresis did not coincide with differentiation in *L. clarki* resistance in *Ae. sierrensis* populations at the microgeographic scale. *Ae. sierrensis* has the capacity to respond to foreign clones of the parasite, but electrophoretic surveys may fail to indicate underlying differences in the parasite resistance of mosquito populations.

KEY WORDS *Lambornella clarki*, *Aedes sierrensis*, parasite resistance, genetic variation, genetic distance

GENETIC VARIATION FOR parasite resistance was examined in the western treehole mosquito, *Aedes sierrensis* (Ludlow), to its parasite, *Lambornella clarki* (Corliss & Coats). Host capacity to evolve resistance to novel parasites or novel parasite genomes is important for understanding the potential for parasitic control of vectors and pests. This capacity depends on the existence and magnitude of additive genetic variation for parasite resistance in the host population (Falconer 1989). Since Huff (1929) first suggested that susceptibility to parasitic infection had a genetic basis, many investigators successfully have selected for increased susceptibility or refractoriness of mosquitoes as intermediate hosts to parasites (Trager 1942, MacDonald 1962, Kilama and Craig 1969, Collins et al. 1986). McGreevy et al. (1974) noted differences in the susceptibility and refractoriness of *Aedes aegypti* (L.) to *Dirofilaria immitis* (Leidy) from 7 different geographically separate locations. Kilama and Craig (1969) found great differences in *Plasmodium gallinaceum* (Brumpt) susceptibility among 10 field-derived strains of *Ae. aegypti*. Both of these studies dealt with strains separated on as great as a continental scale and as small as 290 km. Many investigators have noted rapid response of mosquitos to selection for parasite resistance/susceptibility (*Ae. aegypti*: Bradley et al. 1990, McGreevy et al. 1974, Kilama and Craig 1969; *Anopheles gambiae* (Giles): Collins et al. 1986), often showing substantial responses in a single generation. Herein, we examine genetic variation for parasite resistance between local populations of *Ae. sierrensis* separated by 28 km, examine neutral allelic variation across the same scale by allozyme electrophoresis, and compare variation in resistance with neutral genetic variation on a local scale.

Aedes sierrensis is found along the western coast of North America where it may be a vector of canine heartworm, *D. immitis* (Lavoipierre and Walters 1980). During its immature stages the mosquito co-occurs in some water-filled treeholes with the parasitic tetrahymenid ciliate, *L. clarki* (Washburn et al. 1991). Mosquito larvae release an unknown protein factor into treehole water which cues the transformation of free-living *L. clarki* trophonts to obligately parasitic theronts (Washburn et al. 1988a, Mercer et al. 1989). Other *L. clarki* endoparasites make an obligate transformation to theront form upon excystment or emergence from the host (Washburn et al. 1991). The *L. clarki* theronts seek out a host larva, encyst on the exterior of the cuticle, dissolve a hole in the cuticle, and enter the hemocoel of the host (Corliss and Coats 1976, Washburn et al. 1988b). The endoparasite persists and reproduces in the hemocoel until the

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals and the Institute of Laboratory Animal Resources, National Research Council.

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host dies. If the host survives to adult emergence, the parasite effectively sterilizes the female adult mosquitoes (Egerter et al. 1986). The effect on male reproductive success is unknown. Therefore, the fitness consequences of parasitization are severe for most hosts, especially females.

Resistance to the parasite may occur through several mechanisms. The 1st mechanism is melanization of the encysted theront before it can enter the hemocoel (Corliss and Coats 1976, Washburn et al. 1988a). We have observed partially melanized endoparasites and completely melanized cysts within parasitized hosts (L.E.B., unpublished data). *Ae. sierrensis* also resists infection by preying upon nonendoparasitic *L. clarki* (Washburn et al. 1988a), which are unable to parasitize the mosquito through the gut epithelium. No recovery of the host from successful penetration by the parasite into the hemocoel has been documented.

Materials and Methods

Collection and Establishment of Mosquitoes. One hundred 1st and 2nd instars per month were collected from November 1991 to April 1992, from 2 natural treeholes, 1 infected and 1 uninfected with *L. clarki*, 28.5 km apart in the southern Willamette Valley, Oregon (Fig. 1): treehole 1 (TH1-infected) of Hawley (1985) in Eugene (Lane County) and treehole 3 (HC3-uninfected) near Harrisburg (Linn County). TH1 has been observed to contain *L. clarki* continuously over 12 yr. HC3 is 1 of 4 treeholes in an isolated grove and has not been observed to contain *L. clarki* during the 3 yr of this study (1991–1993). Larval cohorts of 30–35 individuals were raised in petri dishes (160 by 25 mm) filled with distilled water in incubators at a constant 15°C and a photoperiod of 16:8 (L:D) h. Three times per week larvae were fed ad libitum a suspension of ground guinea pig chow in distilled water. Upon pupation, the mosquitoes were placed in a plastic jar filled with distilled water inside a polyethylene adult flight cage separated by population of origin. Cages were maintained in a controlled-environment room with a photoperiod of 18:6 (L:D) h and a smooth sine wave thermoperiod from 12 to 28°C (mean = 21°C) that lagged the photoperiod by 3 h. Pesticide-free raisins were provided as a carbohydrate source for adults. Adult females were allowed to feed to repletion on a human or rat blood host.

An artificial treehole was provided in the form of a blackened glass jar filled with distilled water and a white paper towel wick as an oviposition substrate. The wick was checked for oviposition every 3 d. Eggs were transferred to a plastic petri dish and placed in an incubator at constant 18°C and a photoperiod of 16:8 (L:D) h for 18–21 d to promote embryonation. Following embryonation, eggs were wetted with distilled water, the petri dish was sealed with electrical tape, and the eggs placed in a dark refrigerator at 4°C for 4 mo to promote completion of embryonic diapause.

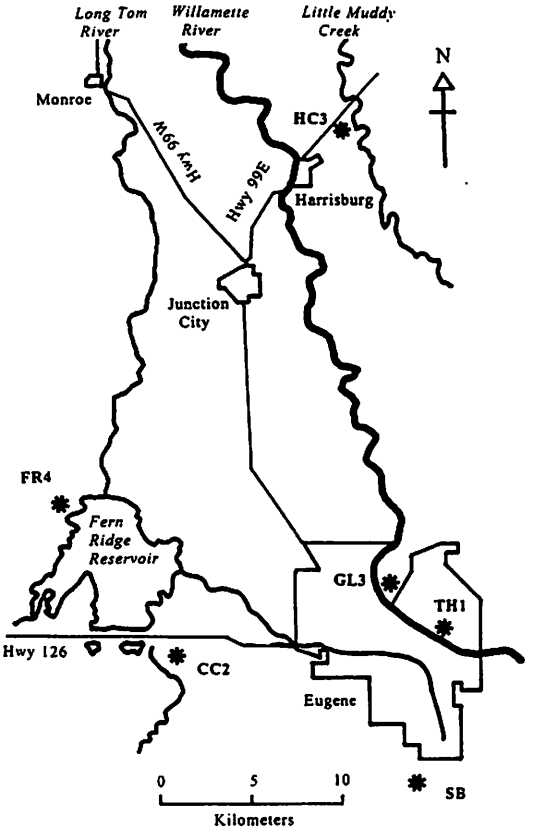

Fig. 1. A map of *Ae. sierrensis* and *L. clarki* sample locations in the southern Willamette Valley, Oregon. Letter designations are given for each population and correspond to the following location identifier for *Ae. sierrensis* samples. CC, Coyote Creek; FR, Fern Ridge Reservoir; GL, Grand Island Loop Road; and HC, Harrisburg Cemetery. SB, Spencer's Butte represents the source of the *L. clarki* clone used in the study. TH represents treeholes in the same location sampled by Hawley (1985).

Cohorts of eggs from each population were selected haphazardly to make up the next generation. After refrigeration, eggs were warmed to 11°C and immersed in an aqueous hatching medium of putrid guinea pig chow and brine shrimp. Of the original HC3 and TH1cohorts, 454 and 719 individuals hatched. All larvae, pupae, adults, and eggs were handled as described above, segregated by population.

Collection and Establishment of the Ciliate Clone. A clone of the parasite *L. clarki* was grown from a single trophont collected from a treehole located 8 km south of TH1 from location SB (Fig. 1). Culture medium consisted of reconstituted treehole water. Tree-hole water was collected at Ashland, OR, in 1984, transported to the laboratory, and evaporated to crystals in porcelain pans. Two grams of crystals were suspended in 1.55 liters

of distilled water. The resulting solution was agitated, boiled for 7–10 min, allowed to cool and the volume restored to 2 liters with distilled water. A single *L. clarki* individual from the above clone was established in a test tube (13 by 100 mm) with 2 ml of medium and 0.05 gm cerophyll leaves from Sigma (St. Louis, MO) as a microbial growth substrate. Before experimentation, transformation capacity of the parasite clone was tested by placing a 0.1-ml sample in 2 ml of medium and exposing the sample to 3 drops of water from a dish containing a high density of freshly hatched 1st-instar *Ae. sierrensis*. Three days later the sample was examined under a dissecting microscope and *L. clarki* transformation from the trophont to theront form confirmed. The Eugene clone used in this study exhibited moderate transmissibility in a preliminary experiment (data not shown).

Heritability of Resistance. Heritability of resistance was tested for 2 populations of *Ae. sierrensis* selected for 1 generation. *L. clarki* clones show reduced theront production after 8 mo and again after 19 mo of axenic culture (Norton et al. 1992). Because of the potential for rapid selection response, the obligate 4 mo embryonic diapause of *Ae. sierrensis*, and the possible decline in clonal infectivity with the passage of time in culture, genetic variation for resistance was assessed after a single generation of selection using one sample from a *L. clarki* infested treehole (TH1) and an *L. clarki*-free treehole (HC3) from 2 different populations.

Fourteen lines of 240 mosquitoes each were derived from each population (TH1 and HC3; 28 total lines), with 7 lines for selection and 7 lines as an unselected control. Each line was initiated by placing a single newly hatched larva into 2 ml of reconstituted treehole water in 24-well tissue-culture plates. Plates were numbered and assigned alternately as selected or control plates. In the selected lines, 5 *L. clarki* trophonts were added to elicit ≈50% parasitization (unpublished data). No *L. clarki* were added to wells of the control plates. Each well was fed 1 drop of a 10% suspension of ground guinea pig chow at the start of the experiment, 2 drops 5 d later, and 1 drop weekly thereafter. The plates were placed in incubators at 15°C and a photoperiod of 16:8 (L:D) h.

At 12–13 d, moribund larvae were examined under a dissecting microscope for evidence of infection. Larvae not displaying evidence of parasitization then were stained with amido black dye (Sigma) for 30 min and examined again for stained *L. clarki* cuticular cysts under the dissecting microscope. Larvae not displaying either stained cuticular cysts or *L. clarki* endoparasites were classified as unparasitized. Larvae with cuticular cysts were classified as parasitized.

At 16–18 d, all larvae in the selected plates were examined under a dissecting microscope for the presence of endoparasites and removed if an infection was detected. Uninfected larvae were returned to the well from which they were removed. Samples of all control lines were also inspected to confirm that *L. clarki* contamination had not occurred.

Once pupation began, pupae were examined at 5-d intervals under a dissecting microscope for signs of infection, sexed, and counted. Any larvae dead at the time of the pupal survey were inspected as before. Uninfected individuals became the selected survivors and were allowed to eclose, mate, bloodfeed, and oviposit as described previously. Control lines were culled haphazardly to match the numbers and sex ratio of the survivors of selection, except that if the selected lines fell below 100 individuals, the control lines were maintained at 100 with an ≈1:1 sex ratio. Eggs were collected and treated as before. Resistance was calculated using all F_1 parasitized immatures as the number of parasitized mosquitoes divided by the number inspected for each line and generation of selection.

Resistance was tested again in the 2nd generation. All individuals in both control and selected lines were exposed to 5 *L. clarki* trophonts from unselected members of the same parasite clone. The numbers of parasitized and unparasitized mosquitoes in both selected and control lines were recorded. Realized heritability of resistance was calculated by treating resistance as a threshold trait, assuming an underlying continuous, normally distributed liability of resistance (Falconer 1989). Response to selection and selection intensity were estimated as in Falconer (1989). Response was calculated treating resistance in the F_2 control lines as equivalent to resistance in the selected F_1 line to account for environmental variation between generations. The minimum heritability detectable in a single generation (h^2), assuming all variation is caused by random genetic drift and none to measurement error, was estimated using Falconer's (1989) approximation $h^2 = 2c/i^2N_e$, where i is the mean selection intensity for the population, $c = 2$ to approximate 2 standard errors and N_e is the mean effective population size in each line within TH1 or HC3 taking into account uneven numbers of males (N_m) and females (N_f) in the postselection parental generation: $N_e = 4N_mN_f/(N_m + N_f)$ (Falconer 1989).

Electrophoretic Survey. Larvae and pupae of *Ae. sierrensis* were collected from 5 treeholes in separate localities, including TH1 and HC3 but excluding SB (Fig. 1), in the spring of 1992 and 1993. Larvae were maintained as described above. Pupae were placed in distilled water in plastic dishes, removed to polyethylene adult flight cages segregated by treehole of origin, and allowed to eclose. Adults were immobilized in a freezer and then either immediately prepared for electrophoresis or frozen at −80°C until tested.

Horizontal starch gel electrophoresis was performed for the presumptive loci phosphoglucomutase (*Pgm*), hexokinase 4 (*Hk4*), hexokinase 3

(*Hk3*), hexokinase 2 (*Hk2*), phosphoglucoisomerase (*Pgi*), esterase (*Est*) and malic enzyme (*Me*) as in Steiner and Joslyn (1979) using the BD buffer/gel system (Lynch 1983). Preparation of the adults consisted of manually homogenizing each individual adult with a glass stirring rod in a well of a 96-well culture plate containing 1 drop of distilled water. A filter paper wick then was used to collect the homogenate for analysis. Gels were run at 200 V and 0.2 amps (EC 420, EC Apparatus, St. Petersburg, FL) until the marker had traveled an established distance. Each gel had samples from >1 treehole to control for differences in mobility. Gels run for the 1993 samples also were run with a few adults of *Aedes triseriatus* (Say) collected from Tall Timbers Research Station, Leon County, Florida, in April of 1993 to compare and confirm electromorph mobilities. Staining followed Steiner and Joslyn (1979). Heterozygote banding patterns were not tested for heritability, but were determined by band intensity and correspondence to known electromorph mobilities for the locus scored.

Data Analyses. Analysis of variance (ANOVA) for the selection experiment used the general linear model (GLM) procedure of SAS (SAS Institute 1985). Percent resistance was normalized by the an arcsine transformation before analysis. Confidence intervals for the proportion parasitized were calculated with correction for continuity (Snedecor and Cochran 1989).

Deviation from Hardy–Weinberg equilibrium was tested by a chi-square test for all gene frequencies using BIOSYS-1, version 1.7 (Swofford and Selander 1981) with correction for small sample size. To measure population differentiation the coefficient of gene differentiation (Nei 1973), $G_{ST} = D_{ST}/H_T$, was calculated for each possible treehole combination in the electrophoretic survey, where D_{ST} is the total difference in individual allele frequencies between localities and H_T is the total population gene diversity. G_{ST} also was calculated from *Ae. sierrensis* allele frequencies reported by Bloem (1991) for comparison. The allele diversity and total heterozygosity were averaged over the 3 presumptive loci to estimate average G_{ST} (Nei 1973).

G_{ST} over all subpopulations was estimated by the average gene diversity for all presumptive loci (Nei 1973). Nei's genetic distance (Nei 1972) and its standard error (Nei and Roychoudhury 1974) was calculated from the data to allow pairwise comparison with other studies and to measure population differentiation. Correlation of genetic distance (G_{ST} or Nei's) with geographic distance was tested using a Mantel test (Mantel 1967, Manly 1991) with 1,000 randomizations to account for possible nonindependence of genetic distance estimates.

Results

Resistance. *Ae. sierrensis* resistance to a foreign clone of *L. clarki* was moderate to high. Two-way ANOVA of resistance, with generation and population as treatments, showed that resistance was lower in the F_1 (53%) than the F_2 (85%) generations ($F = 31.47$; df = 1, 36; $P < 0.001$; Fig. 2) and that resistance was higher in the TH1 than in the HC3 population ($F = 8.91$; df = 1, 36; $P = 0.005$; Fig. 2); there was no generation by population interaction ($F = 0.29$; df = 1, 36; $P = 0.60$). Two-way ANOVA of resistance in the F_2 with population and selection (selected, control) as treatments again showed that resistance was higher in the TH1 than in the HC3 population ($F = 10.59$; df = 1, 22; $P = 0.004$; Fig. 2), but resistance did not differ between the selected and control lines ($F = 0.22$; df = 1, 22; $P = 0.65$; Fig. 2); there was no population by selection interaction ($F = 0.01$; df = 1, 22; $P = 0.93$). The realized selection differential imposed by 1 generation of selection was greater in the HC3 (0.75 ± 0.09 SE) than in the TH1 (0.42 ± 0.13 SE) population. Assuming zero measurement error, our methodology could have detected minimum non-zero heritabilities of 0.09 and 0.20 in these respective populations. In fact, the realized heritabilities of resistance (±1.96 SE) were 0.027 ± 0.812 in the TH1 population and 0.212 ± 0.219 in the HC3 population; both were nonsignificant, in accordance with ANOVA. These results show that resistance to parasitism was consistently higher in the TH1 than the HC3 population, was higher in the F_2 than the F_1, but did not respond to one generation of selection in either population.

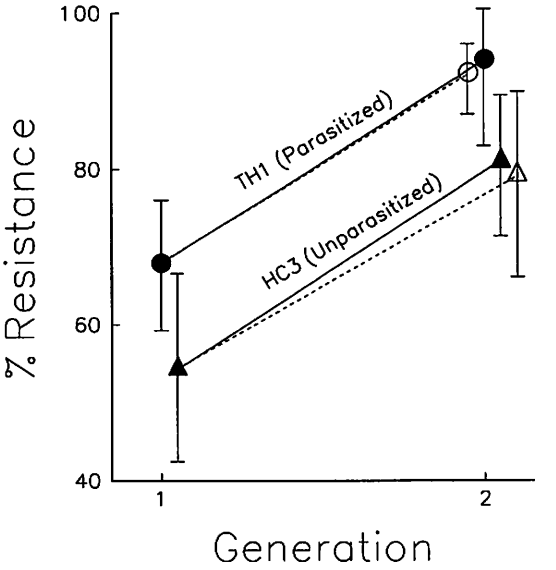

Fig. 2. The response of resistance to artificial selection in *Ae. sierrensis*. Percent resistance is the back-transformed population mean of arcsine transformed proportions resisting parasitization in each line shown. Open symbols are control line population means in the 2nd generation of the selection experiment. Error bars are ±2 SE.

Table 1. Allele frequencies at 3 loci for 5 populations of *Ae. sierrensis* and 1 populations of *Ae. triseriatus* (sample size in parentheses)

Rf	Populations					
	FR-4	TH-1	HC-3	GL-3	CC-2	*Ae. triseriatus*
			Pgm			
No.	(55)	(52)	(19)	(11)	(21)	(22)
1.36	—	—	—	—	—	0.227
1.18	0.018	0.115	0.053	—	0.024	0.227
1.09	—	—	—	—	—	0.091
1.00	0.945	0.827	0.947	1.000	0.976	0.432
0.91	0.009	0.038	—	—	—	0.023
0.82	0.018	0.019	—	—	—	—
0.72	0.009	—	—	—	—	—
			Hk4			
No.	(36)	(67)	(40)	(19)	(13)	(11)
1.03	—	—	0.025	—	—	—
1.00	1.000	0.993	0.962	1.000	1.000	—
0.97	—	0.007	0.013	—	—	—
0.96	—	—	—	—	—	0.091
0.93	—	—	—	—	—	0.682
0.90	—	—	—	—	—	0.227
			Hk3			
No.	(34)	(35)	(26)	(9)	(13)	(11)
1.06	0.074	—	—	—	0.077	—
1.00	0.926	1.000	1.000	1.000	0.923	—
0.93	—	—	—	—	—	0.409
0.88	—	—	—	—	—	0.591

The selected lines showed lower resistance in the F_1 compared with both selected and unselected lines in the F_2 ($F = 24.22$; df = 1, 34; $P < 0.001$), which is consistent with a decrease in transmissibility of the parasite clone over time. Norton et al. (1992) reported a reduction in the transformation ability of *L. clarki* trophonts after 8 and 19 mo of axenic culture. The clone used here had been maintained in laboratory culture for ≈14 mo when exposed to *Ae. sierrensis* larvae in the 2nd generation. Reduced transformation ability in the clone used in this study is indicated by these results and may account for some of the inability to distinguish a response to selection. Differences between the populations persisted despite the apparent loss of transformation capacity. This persistence in a common environment indicates that the difference in resistance is a genetic and not an environmental effect.

Electrophoresis. *Est*, *Pgi*, *Hk2*, and *Me* produced variable staining patterns that defied reliable interpretation and therefore were rejected for further analysis. Heterozygotes of *Pgm*, *Hk4* and *Hk3* were distinguished from nongenetic artifacts by staining intensity and band mobility. Three of 5 *Ae. sierrensis* populations were polymorphic at the *Pgm* locus, none at *Hk4*, and 2 at *Hk2* at the 5% level (Table 1). *Pgm* had 7 alleles, *Hk4* had 3 alleles, and *Hk3* had 2 alleles (Table 1). *Ae. triseriatus* was polymorphic at all 3 loci. *Ae. sierrensis* and *Ae. triseriatus* can be distinguished by the differing mobilities for *Hk4* and *Hk3* (Table 1). There were no significant deviations from expected Hardy–Weinberg proportions for any single locus in the 5 *Ae. sierrensis* populations (χ^2 = 0–2.135, df = 1–10, $P > 0.05$) or the *Ae. triseriatus* sample (χ^2 = 2.0–3.098, df = 1, $P > 0.05$). Total chi-square did not show a significant departure from Hardy–Weinberg expectations overall for *Ae. sierrensis* (total = 2.57, df = 14, $P > 0.05$). The *Ae. triseriatus* sample did depart significantly from Hardy–Weinberg expectations overall (total = 7.694, df = 2, $P < 0.05$). Small sample size for the *Ae. triseriatus* population could be responsible for the overall deviation from random mating expectations. There is no substantial evidence, however, that mating is assortative in the *Ae. sierrensis* populations. Therefore, random mating was assumed for the balance of the analyses.

Electrophoresis showed a low level of differentiation among populations of *Ae. sierrensis* (Fig. 3). G_{ST} was not correlated with geographic distance between populations (Mantel's test: $\beta[0] = 0.088$, $\beta[1] = -0.016$, $P > 0.05$; Fig. 3A). Mean ± 1.96 SE $G_{ST} = 0.055 \pm 0.020$ between populations. There was less differentiation at these 3 loci between TH1 and HC3 (infested versus uninfested with *L. clarki*: $G_{ST} = 0.043 \pm 0.022$) over a distance of 28.5 km than between TH1 and GL3 (both infested with *L. clarki*: $G_{ST} = 0.126 \pm 0.070$) over a distance of 5.1 km. *Ae. triseriatus* had a mean $G_{ST} = 0.643 \pm 0.018$ compared with all *Ae. sierrensis* populations.

All *Ae. sierrensis* populations combined had a mean ± 1.96 SE Nei's genetic distance of 0.003 ± 0.001. *Ae. triseriatus* had a mean Nei's genetic distance of 1.534 ± 0.027 from all *Ae. sierrensis* populations surveyed, consistent with substantial ge-

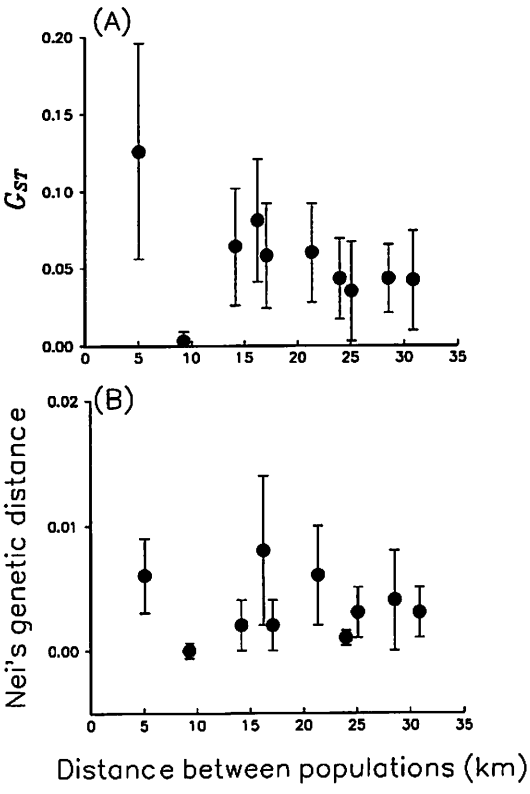

Fig. 3. Microgeographic genetic differentiation in *A. sierrensis* as estimated (A) by G_{ST} (Nei 1973) or (B) by genetic distance (Nei 1972). Error bars are ±2 SE.

netic differences between species. Whereas, the *Ae. triseriatus* population deviated from random mating expectations overall, the distance noted here may be largely attributable to the differences at *Hk4* and *Hk3* and should be somewhat independent of the influence of deviations from Hardy–Weinberg expectations. The genetic distances between *Ae. sierrensis* treehole subpopulations ranged from 0 to 0.008 (Fig. 3B). Differentiation was not correlated with distance between locations or treeholes within a location (Mantel's test: β [0] = 0.004, β[1] < −0.001, $P > 0.05$; Fig. 3B).

Discussion

Our results demonstrated that *L. clarki* resistance in *Ae. sierrensis* has a genetic basis. TH1 and HC3 populations differed in resistance and this difference persisted through 2 generations in a common environment during the selection experiment. Greater resistance was found in the *L. clarki*-infested population (TH1) than in the *L. clarki*-free population (HC3). This difference in resistance establishes genetic differences between populations and provides consistent, but not conclusive, evidence for the role of selection in parasite resistance because there was no independent replication of tree-holes within treatments (*L. clarki*-infested versus *L. clarki*-free). Despite persistant differences in resistance between populations, there was no demonstrable evidence for heritable variation within either the *L. clarki*-free or the *L. clarki*-infested population. Indeed, variation in resistance between generations (an unexplained environmental effect) was clearly much greater than variation in resistance between selected and unselected lines within either population.

Lower resistance in F_1 selected lines compared with F_2 controls indicated either that the infection capacity of the parasite clone had decreased with time or that, by chance, the controls were a subsample of more highly resistant genotypes. The former is consistent with the observation of Norton et al. (1992) and the latter seems unlikely given the degree of replication and size of the selected and control lines in the experiment. Either cause, however, could have prevented the detection of a response to selection in this experiment. Other environmental factors are unlikely given the number of generations the lines were reared in a common environment (4 including the 2 generations before the experiment). The increased resistance seen in F_2 controls may be further evidence of a deteriorating infection capacity in cultured *L. clarki*. Any use of *L. clarki* as a biological control would have to overcome this temporal constraint.

The capacity of *Ae. aegypti* to rapidly develop high resistance or susceptibility to parasitism following selection has been well established (Trager 1942, Kilama and Craig 1969, Bradley et al. 1990). Kilama and Craig (1969) noted an "immediate" response to selection for resistance to *P. gallinaceum* and complete resistance after 6 generations of selection. Trager (1942) noted the greatest increase in *Plasmodium lophurae* (Coggeshall) in the 1st generation of selection. High resistance to the novel parasite *D. immitis* followed selection in the laboratory for 3 generations (Bradley et al. 1990). Rapid responses to selection are possible in mosquito hosts, yet no such response was observed in *Ae. sierrensis*. In contrast, our results indicate that parasite resistance is genetic but does not respond rapidly to selection within populations of *Ae. sierrensis*. The implication for host–parasite coevolutionary potential is that *Ae. sierrensis* can evolve resistance, but not rapidly.

Despite the rapid selection response observed in other mosquitoes, a single generation of selection also may prevent detectable differences between selected lines and controls. Although more generations of selection would have been desirable, the probability of deteriorating parasite clonal infection ability (Norton et al. 1992) and the necessity of a 4-mo obligate embryonic diapause to recover all genotypes presented a barrier to long-term selection experiments in the *L. clarki*/*Ae. sierrensis* parasite/host system.

Bloem (1991) surveyed 10 electrophoretic loci from 9 populations of *Ae. sierrensis* as widely sep-

arated as San Diego, CA, and Seattle, WA (≈2,000 km), and concluded that there was substantial gene flow and "genetic cohesiveness" among *Ae. sierrensis* populations. Based on Bloem's (1991) reported allele frequencies, the mean G_{ST} is 0.112 (Nei 1973) across much of the range of *Ae. sierrensis*, which is roughly twice our estimate at the microgeographic scale with 3 presumptive electrophoretic loci. This reduced differentiation is consistent with the broader sample range of Bloem's study and indicates that gene flow is probably higher in our more local populations, as might be expected based on prior dispersal evidence (see Bennet 1980). The fairly consistent level of differentiation observed between these populations at the local scale (Fig. 3) indicates that Bloem's characterization of gene flow and population genetic structure hold at the microgeographic scale as well. Thus, genetic distance and G_{ST} between closer populations are generally as high as among more distant populations separated by up to 30 km regardless of *L. clarki* presence or absence (Fig. 3).

Differentiation between *Ae. sierrensis* and *Ae. triseriatus* is consistent with their taxonomic classification. *Hk4* and *Hk3* serve as presumptive electrophoretic loci that can distinguish between the species based on electromorph mobility. The difference between species is consistent with the substantially lower estimates of *Ae. sierrensis* intraspecific differentiation. The estimated G_{ST} between these species is roughly 5 times that reported by Bloem (1991) within *Ae. sierrensis* alone.

The probable difference in selection pressure for parasite resistance between *L. clarki*-free and *L. clarki*-infested *Ae. sierrensis* populations was not reflected by differentiation in the 3 neutral loci analyzed. Other *L. clarki*-infested populations (GL3), which have been presumably subject to selection, show greater differentiation from TH1 than the presumably unselected population, HC3, despite the population differences in the selected trait of *L. clarki* resistance. Therefore, neutral genetic differentiation did not accurately reflect the level of differentiation of parasite resistance at the microgeographic scale. A larger sample of electrophoretic loci might resolve this disparity; however, Bloem's (1991) results suggest that the potential for determining greater differentiation of local populations with further loci might be low. Electrophoretic surveys of *Ae. sierrensis* populations are inadequate to estimate either existing or potential variation in *L. clarki* resistance.

The differences in parasite resistance observed between populations derived from infected and uninfected treeholes indicate that parasite resistance has a genetic basis in *Ae. sierrensis*. The lack of a significant response to 1 generation of selection in the laboratory indicates that the heritability of resistance, though probably nonzero, is low. The relative level of neutral genetic differentiation observed among local populations was a poor indicator of genetic variation in *L. clarki* resistance among populations of *Ae. sierrensis*.

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