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ALLOZYME DIVERSITY IN *ANOPHELES QUADRIMACULATUS* (SENSU STRICTO) POPULATIONS IN NORTHEASTERN ARKANSAS

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ABSTRACT. A comparison of electrophoretically detectable isozyme differences in 6 populations of *Anopheles quadrimaculatus* (sensu stricto) from northeastern Arkansas was undertaken to test the hypothesis that microgeographic variation in habitat types was promoting significant within- and between-population genetic diversity. Genetic heterogeneity within populations was substantial, with all of the enzyme loci examined having 2–7 alleles and average levels of polymorphisms per population between 54.5 and 72.7%. Heterozygotes made up an average over all loci of between 20.6 and 24.8% of the individuals examined. Only weak evidence was found for gametic disequilibrium between pairs of loci. Neither F-statistic nor genetic distance analysis suggested interpopulation divergence. The F_{ST} value averaged over loci was 0.190. All Nei distances for pair-wise population comparisons were greater than 0.010, which was much lower than published values from comparisons between populations belonging to different species of the complex. Divergence was not significantly correlated to either geographic distance or habitat type. Examination of the results suggests that little genetic divergence has occurred between populations of *An. quadrimaculatus* in northeastern Arkansas, possibly because of the dispersal ability and low level of discrimination between oviposition sites exhibited by this species.

KEY WORDS Mosquitoes, isozymes, electrophoresis, allozyme polymorphism, genetic differentiation

INTRODUCTION

In northeastern Arkansas, the species of the *Anopheles quadrimaculatus* (Say) complex are a major component of the insect fauna, a major nuisance, and a potentially dangerous vector of diseases such as malaria and parasites such as dog heartworm during the summer months. The primary species of the complex in northeastern Arkansas is *An. quadrimaculatus.* Lanzaro et al. (1990) also reported the occurrence of *An. smaragdinus* Reinert at Stuttgart, AR, but its presence in the northeastern part of the state has not been confirmed. None of the other species of the complex have been recorded from Arkansas, although they have been found in neighboring regions of Tennessee, Mississippi, and Louisiana (Seawright et al. 1992, Reinert et al. 1997, Rutledge and Meek 1998).

Within the complex, the species appear to have diverged, at least in part, because of differences in their larval habitat (Reinert et al. 1997). The larvae of *An. quadrimaculatus* s.s. are found in diverse habitats that have either open or shaded permanent water. This is the primary sibling species of the complex believed to be associated with rice fields, irrigated cotton and soybeans, and ditches in northeastern Arkansas. Larvae of *An. smaragdinus* are found in permanent swamp water that has moderate emergent vegetation shaded by a forest canopy. The other species of the complex have been found in temporary pools that form in or near densely shaded swampy areas or slow-moving streams.

Northeastern Arkansas contains the same diverse habitat types locally that seem to have encouraged the divergence of the species of this complex generally. The land is predominantly flat alluvial plain of the Mississippi River delta. The natural bottomland hardwood forest has been largely replaced by agriculture, with rice, corn, cotton, and soybeans being the major crops. These agricultural fields offer little shade but contain both permanent and transient oviposition sites. The eastern portion of the region contains substantial bald cypress swampland with heavy shade, emergent vegetation, and algal growth. Through the center of the region, Crowley's Ridge rises up to 61 m above the surrounding plain, forming a potential barrier up to 16 km wide to free movement of mosquitoes. However, its natural streams and man-made lakes surrounded by upland hardwood forest may provide another habitat to support genetic diversity within the complex, or even the existence of yet unrecognized taxa. The presence of *An. diluvialis* Reinert within the broader range of the genetically similar *An. inundatus* Reinert suggests that speciation may have occurred at a microgeographic scale (Narang et al. 1990) similar to that in northeastern Arkansas.

The purpose of this study was to compare the electrophoretically detectable enzyme phenotypes of *An. quadrimaculatus* from localities in the region that had different habitat types. Differing electrophoretic patterns were the 1st evidence for the existence of more than 1 taxon within *An. quadrimaculatus* s.l. (Lanzaro 1986). Previous isozyme analyses of this species in northeastern Arkansas (Bearden 1996) had revealed suggestive differences for several enzymes. The current study sought to confirm those results and to determine the extent of genetic differentiation between populations. The hypothesis of divergence within the species *An. quadrimaculatus* in northeastern Arkansas was to be tested against the null hypothesis of no divergence. The data were then to be examined in light of corollary hypotheses that genetic divergence was correlated with particular habitat types or that it reflected divergence by distance. Finally, the data

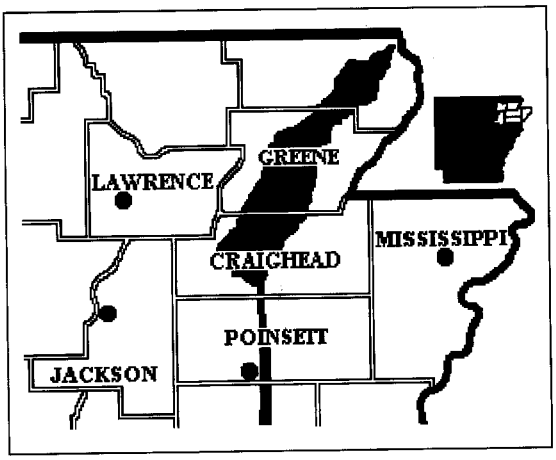

Fig. 1. Collection sites for *Anopheles quadrimaculatus* in northeastern Arkansas. Gray area is Crowley's Ridge.

were tested for evidence of new, previously undescribed species belonging to the complex. The alternative of all these hypotheses, no significant genetic divergence, is the condition predicted if substantial gene flow was present, resulting from the good dispersal powers and use of many types of oviposition sites by the adult mosquitoes.

MATERIALS AND METHODS

The mosquito samples were collected during the summer of 2000 from 6 locations in Arkansas: Craighead County, collected in a housing subdivision of southwestern Jonesboro (35°48.1′N, 90°40.8′W), July 25 and 30; Jackson County, collected at the bath house of Jacksonport State Park (35°38.2′N, 91°18.7′W), August 2; Mississippi County, collected on the road to Mallard Lake 0.5 km NE of intersection with State Route 18 (35°51.0′N, 90°07.3′W), August 5; Poinsett County, collected near rice fields 10 km S of Harrisburg on State Route 1 (35°27.6′N, 90°44.8′W), August 8; Greene County, collected at the Information Center of Crowley's Ridge State Park (36°02.6′N, 90°39.9′W), August 9; and Lawrence County, collected at the picnic grounds of Lake Charles State Park (36°04.0′N, 91°09.2′W), September 28 (Fig. 1). The collecting sites in Craighead County and Greene County were on Crowley's Ridge. The Mississippi County site was in a marshy lowland habitat. The Poinsett County site was in a rice field. The Lawrence County and Jackson County sites were more distant localities resembling the Greene County and Poinsett County sites, respectively.

Mosquitoes were collected immediately after dark by sweep netting as they were attracted to the collector standing in the headlights of an automobile. Each collection, consisting of more than 100 *Anopheles,* was placed into a cooler with ice for transport to the laboratory. There, the mosquitoes were sedated by cooling and the *Anopheles* were separated from other species. The *Anopheles* were placed into cryogenic tubes and stored in a freezer at −70°C until submitted to electrophoresis. By collecting the mosquitoes by using the same method and over a short period of time, it was expected that only *An. quadrimaculatus* s.s. would be taken and that collection of other members of the complex would be minimized.

The enzyme systems mannose-6-phosphate isomerase (MPI, 5.3.1.8), isocitrate dehydrogenase (IDH, 1.1.1.42), 3-hydroxybutyrate dehydrogenase (HAD, 1.1.1.30), and glycerol-3-phosphate dehydrogenase (GPD, 1.1.1.8) were examined by using 12% starch gels made with the CA8 buffer system of Steiner and Joslyn (1979). Aspartate aminotransferase (AAT, 2.6.1.1), glucose-6-phosphate isomerase (PGI, 5.3.1.9), phosphoglucomutase (PGM, 5.4.2.2), and malate dehydrogenase (MDH, 1.1.1.37) were examined by using 12% starch gels and the NAM7 buffer system of Clayton and Tretiak (1972).

The procedures used to prepare the mosquitoes, load the gels, and perform the electrophoresis were substantially those described by Narang et al. (1989a, 1989b, 1989c). After bromphenol blue markers had migrated 10 cm, the gels were sliced and each slice was stained for a different enzyme system by using the histological procedures of Steiner and Joslyn (1979). When the banding patterns had developed, the gels were scored and the migration distances of the bands were recorded.

In the electrophoretic keys to the species of the *An. quadrimaculatus* complex, relative migration distances were calculated compared to a standard laboratory strain (the Q2 strain of *An. quadrimaculatus*). That strain was not available. The assumption had been made that the prevalent sibling species in Arkansas populations was *An. quadrimaculatus* s.s. and that migration distances and allele frequencies did not differ substantially from those observed at Stuttgart and Beebe, AR (Lanzaro et al. 1990). This assumption was justified on 3 grounds. First, Narang et al. (1989a) examined the Stuttgart population after Lanzaro (1986) and Lanzaro et al. (1990) and found only *An. quadrimaculatus* in the population; 2nd, examination of the frequency data reported by Narang et al. (1989b, 1989c) suggested that, for all of the key systems, the same alleles were predominant in populations of *An. quadrimaculatus* irrespective of the geographic source; and 3rd, when relative migration (R_f) values for the alleles of the different enzymes examined in the current study were calculated, the patterns matched those reported in the 4 major sources (Lanzaro et al. 1990; Narang et al. 1989a, 1989b, 1989c). Except for PGI, which was run on a different system than used by Narang et al. (1989a, 1989b, 1989c), the enzyme locus designations and allele identifiers used here for en-

zymes that distinguish species of the complex will be the same as those given in the keys.

Data were summarized and statistical tests were performed with BIOSYS (Swofford and Selander 1981) and BIOSYS2 (Black and Krafsur 1985a, 1985b). Chi-square goodness-of-fit tests were performed on the enzyme phenotype data of all enzymes in the 6 populations to test for Hardy–Weinberg equilibrium. Heterogeneity of phenotype proportions among populations was tested by contingency table analysis. The sequential Bonferroni method (Rice 1989) was used to adjust significance values for multiple comparisons in both these procedures. Nei distance statistics (Nei 1978) and F-statistics (Wright 1978) were used to measure the degree of genetic divergence between populations. To determine if any divergence was due simply to geographic distance, F_{ST} statistics were calculated for pairs of populations and the matrix of $F_{ST}/(1 - F_{ST})$ was produced. This linearized genetic distance matrix was then compared to a matrix of the geographic distances by using a Mantel test, which is a nonparametric procedure used to measure the correlation between 2 matrices (Sokal and Rohlf 1995). The probability that the resulting correlation differed from zero was computed by using 1,000 permutations. The test also was run between Nei distance statistics and geographic distances. Phenograms were prepared from the F_{ST} statistics and the Nei distance measures to determine if populations clustered according to their habitat type or geographic proximity. An analysis of linkage disequilibrium between pairs of the loci was performed to determine if evidence existed for genetic divergence within unrecognized members of the species complex (Black and Krafsur 1985b).

RESULTS

Electrophoretic phenotype data were obtained for 11 enzyme loci from 576 mosquitoes distributed among the 6 populations of *An. quadrimaculatus* (Table 1). All of the loci were polymorphic for at least 2 alleles. The greatest number of alleles (7) was found for MPI-1; PGI and MDH-C had the fewest alleles (2). Only 2 individuals had enzyme phenotypes that identified them as members of other species of the complex by using the biochemical keys (Narang et al. 1989a, 1989b, 1989c; Reinert et al. 1997). Both keyed to *An. smaragdinus*. Data for these 2 individuals were removed from further analyses.

The sample from Mississippi County was the most variable, with an average of 3.8 alleles per locus, an average 72.7% of the loci polymorphic (with loci being considered polymorphic if the most common allele occurred at a frequency of less than 0.99), and a mean heterozygosity of 0.248 ± 0.056 (obtained by calculating the proportion of individuals that were heterozygous for alleles at each locus and averaging over all loci). The sample from Lawrence County had the fewest alleles per locus (2) and lowest percentage of polymorphic loci (54.5%), both low because of the small size of the sample (11). Craighead County had the lowest level of heterozygosity (0.206 ± 0.046).

In all counties, the average heterozygosities were less than those expected at Hardy–Weinberg equilibrium. However, only for the enzymes HAD-3 and MPI-1 were departures from equilibrium statistically significant ($P < 0.01$) by Chi-square goodness-of-fit Analysis modified by sequential Bonferroni procedures (5 of 6 samples for both loci).

The enzyme HAD-3 also exhibited significant interpopulation differences, as determined by contingency table analysis ($P < 0.05$) after Bonferroni modification. The greatest contribution to this significant departure was from homozygote overrepresentation and heterozygote underrepresentation in most samples. The enzyme MDH-A also produced significant chi-square values ($P < 0.05$) in contingency table analyses, but the cause appeared to be more heterozygotes and fewer homozygotes than expected in the samples from Craighead and Greene counties.

The F-statistics were calculated from allele frequency data by using the FSTAT routine of BIOSYS (Swofford and Selander 1981) with samples treated as subpopulations. The Lawrence County sample was not included in any analyses comparing populations because of its small size. The mean value of F_{ST} averaged over enzyme loci was 0.019. The F_{ST}-values for the individual loci ranged from a high value of 0.031 for HAD-3 to a low value of 0.007 for PGI and AAT-A. The mean values of F_{IS} and F_{IT} were 0.160 and 0.176, respectively. Values of F_{IS} varied from 0.340 for MPI-1 to −0.004 for PGM. The high value for F_{IT} was 0.347 for MPI-1 and the low value was −0.008 for MDH-C and αGPD.

Pairwise F_{ST}-values were small, averaging 0.006. The lowest F_{ST}-values were 0.003 for comparisons of Craighead County to Jackson County and Mississippi County to both Greene and Poinsett counties. The largest value was 0.013 for the comparison of Poinsett County to Jackson County.

The Nei distance measures (D) between pairs of populations were calculated from the allele frequencies by using the SIMDIS subroutine of BIOSYS (Swofford and Selander 1981). The largest value observed was from the comparison of Greene and Lawrence counties, D = 0.010 (where 0 represents no difference between the populations and 1.0 represents complete dissimilarity). Two comparisons, Craighead County to Jackson County and Poinsett County to Mississippi County, gave values of D = 0.0, whereas 3 comparisons, Craighead County to Mississippi County and Greene County to both Poinsett and Mississippi counties, gave values of D = 0.001.

Phenograms prepared by hand by using the unweighted pair group method with arithmetic mean

(UPGMA) procedure for both the pair-wise F_{ST}-statistics and the Nei distance values both revealed 2 population clusters, 1 containing Craighead and Jackson counties (D = 0.0; F_{ST} = 0.003), and a 2nd containing Poinsett, Mississippi, and Greene counties (D = 0.001; F_{ST} = 0.003). The 2 clusters were separated by an average distance of D = 0.004 (F_{ST} = 0.008).

Mantel tests comparing a matrix of geographic distances between collecting sites and a matrix either of F_{ST}-statistics linearized by using the formula $F_{ST}/(1 - F_{ST})$ or the Nei distance statistics yielded Z values of 1.86 and 3.97, respectively. These Z values corresponded to correlation coefficients of −0.263 and −0.272, respectively. Neither value was significantly different from 0.0 ($P > 0.05$), based on distributions generated by 1,000 permutations of the geographic matrix. Examination of these results indicated no significant relationship between genetic divergence and geographic distance.

Tests for gametic (linkage) disequilibrium performed by the LINKDIS program in BIOSYS2 (Black and Krafsur 1985b) revealed 51 significant interlocus associations for 132 pairwise tests ($P < 0.05$). Only 8 of these associations, involving 6 loci and 4 populations, remained significant after application of a Bonferroni correction. Because this proportion of significant tests (2.4%) is less than the type I error rate of the tests, the evidence for disequilibrium must be viewed as weak.

DISCUSSION

Despite the presence in northeastern Arkansas of the habitat variability that has given rise to speciation events on the large scale of the southeastern United States and on a microgeographic scale in northern Florida, examination of the electrophoretic data offers little evidence of the presence of genetically distinct populations in northeastern Arkansas. Only 2 of 576 mosquitoes may have been from a species of the complex other than *An. quadrimaculatus*. However, in both instances, the phenotypes could have occurred in *An. quadrimaculatus* by combinations of infrequent alleles that have been observed in the species in this and previous studies.

Certainly, the genetic heterogeneity required to detect divergence is present in the region. All of the loci carried more than 1 allele and for some, such as MPI-1 and HAD-3, polymorphism was substantial. At loci known to exhibit allele frequency differences between recognized members of the *An. quadrimaculatus* complex, alternative alleles were present and at substantial frequencies. However, the similarities in the frequencies revealed by contingency table, F-statistics, and Nei distance analyses suggest that between-population divergence was minimal.

Bearden (1996) reported significant heterozygote deficiencies in HAD-3 and MDH-C for populations from northeastern Arkansas. Similar heterozygote deficiencies were observed in the present study. The enzyme HAD-3 had alleles that differed both in migration and in strength of expression, and the alleles with weak expression had the same migration rates as corresponding alleles with strong expression. Some weak/strong-expression heterozygotes were scored as strong-expression homozygotes, which inflated the numbers in the latter class while decreasing the numbers in the former. Some of the significant chi-square tests for HAD-3 were because of these misclassifications. No significant departures from Hardy–Weinberg frequencies were observed for MDH-C. The techniques used by Bearden (1996) were different from those in this study. Perhaps the 2 electrophoretic conditions differed in their abilities to resolve small variations in allozyme migration.

Bearden (1996) also reported significant heterozygote deficiencies in more than 1 county for MPI-1, IDH-1, αGPD, and PGI. Of these loci, only MPI-1 was observed to be significantly different from Hardy–Weinberg equilibrium. Bearden (1996) reported only 3 alleles for MPI-1 and IDH-1, whereas the present study revealed 6 alleles for MPI-1 and 4 alleles for IDH-1. If homozygotes and heterozygotes for unrecognized alleles had been counted as homozygotes for 1 of the recognized alleles in Bearden (1996), homozygote classes would have been overestimated and heterozygote classes would have been underestimated for both IDH-1 and MPI-1, yielding the significant chi-square tests. However, alleles at MPI-1 distinguish *An. maverlius* Reinert from the other members of the *An. quadrimaculatus* complex. Alleles of MPI-1 differ appreciably in frequency between *An. quadrimaculatus, An. inundatus,* and *An. maverlius* (Narang et al. 1989a, 1989b, 1989c). Therefore, the significant departures from equilibrium observed for MPI-1 in both of these studies bear further investigation.

For both αGPD and PGI, Bearden (1996) observed heterozygote deficiency when the alternative alleles at the locus both occurred at frequencies greater than about 0.2. In the present study, frequencies of less common alleles were not greater than 0.05 for either of these loci. This observation suggests the possibility that too few heterozygotes or alternative homozygotes were observed to detect the effects of inbreeding. The deficiencies previously observed in certain populations for αGPD and PGI justify further study.

The F-statistic analysis revealed little interpopulation divergence. The F_{ST} measures the probability that at some earlier time 2 genes in different samples arose from the same gene in the total population represented by all of the samples. Therefore, F_{ST} is a measure of the genetic divergence between populations. The F_{IS} is the probability, measured relative to other genes in that local population, that 2 genes within an individual are descendants of the

Table 1. Frequencies of alleles observed at 11 loci examined in *Anopheles quadrimaculatus* (sensu lato) collected from 6 counties in northeastern Arkansas. The most frequent allele at each locus is in bold type.

R_f[1]	Craighead	Poinsett	Greene	Mississippi	Jackson	Lawrence
			Mannose-6-phosphate isomerase, locus 1			
125	—	—	—	—	0.025	—
106	0.005	0.028	0.006	0.029	—	—
100	0.403	0.438	0.351	0.340	0.337	0.500
92	**0.495**	**0.445**	**0.523**	**0.539**	**0.563**	**0.455**
87	0.073	0.049	0.092	0.058	0.050	0.045
82	0.019	0.042	0.011	0.034	0.025	—
78	0.005	—	0.017	—	—	—
n	103	72	87	103	40	11
			Isocitrate dehydrogenase, locus 1			
110	0.069	0.105	0.044	0.101	0.042	0.136
105	—	0.005	—	—	0.010	—
100	**0.726**	**0.705**	**0.766**	**0.745**	**0.740**	**0.500**
85	0.205	0.185	0.190	0.154	0.208	0.364
n	144	100	126	104	48	11
			Isocitrate dehydrogenase, locus 2			
181	0.003	0.005	—	0.005	—	—
162	0.039	0.055	0.031	0.048	0.086	—
136	**0.924**	**0.919**	**0.937**	**0.933**	**0.886**	**1.000**
100	0.030	0.020	0.031	0.014	0.028	—
62	0.003	—	—	—	—	—
n	152	99	127	104	70	11
			3-Hydroxybutyrate dehydrogenase, locus 3			
208	—	—	—	0.005	0.006	—
160S	0.339	0.192	0.175	0.227	0.395	0.227
160W	0.084	0.086	0.078	0.096	0.093	0.182
100S	**0.503**	**0.682**	**0.680**	**0.591**	**0.426**	**0.454**
100W	0.067	0.035	0.058	0.076	0.080	0.136
45	0.007	0.005	0.010	0.005	—	—
n	149	99	103	99	81	11
			Aspartate aminotransferase, cathodal locus			
300	—	—	—	0.006	—	—
228	—	0.006	—	0.011	—	—
200	0.372	0.421	0.359	0.427	0.360	0.409
100	**0.597**	**0.562**	**0.618**	**0.539**	**0.628**	**0.591**
50	0.031	0.011	0.023	0.017	0.012	—
n	98	99	110	89	82	11
			Aspartate aminotransferase, anodal locus			
138	0.005	—	—	—	—	—
122	0.027	0.029	0.022	0.017	0.006	0.045
100	**0.957**	**0.931**	**0.943**	**0.933**	**0.974**	**0.909**
80	0.011	0.034	0.022	0.039	0.019	0.045
74	—	0.006	0.009	0.006	—	—
60	—	—	0.004	0.006	—	—
n	93	87	114	89	78	11
			Glucose-6-phosphate isomerase			
100	**0.990**	**0.977**	**0.966**	**0.972**	**0.981**	**1.00**
80	0.010	0.023	0.034	0.028	0.019	—
n	105	111	89	89	81	11
			Phosphoglucomutase			
125	0.095	0.116	0.068	0.105	0.066	—
100	**0.832**	**0.836**	**0.821**	**0.851**	**0.869**	**0.773**
75	0.063	0.034	0.100	0.035	0.066	0.227
60	0.010	0.014	0.011	0.009	—	—
n	95	73	95	57	61	11
			Malate dehydrogenase, cathodal locus			
100	**0.986**	**0.995**	**1.000**	**0.978**	**0.994**	**1.000**
82	0.014	0.005	—	0.022	0.006	—
n	108	91	111	89	81	11

Table 1. Continued.

R_f[1]	Craighead	Poinsett	Greene	Mississippi	Jackson	Lawrence
			Malate dehydrogenase, anodal locus			
150	0.004	0.045	0.008	0.011	0.048	—
125	0.038	0.045	0.066	0.039	0.062	—
100	**0.899**	**0.837**	**0.795**	**0.831**	**0.822**	**1.000**
60	0.059	0.074	0.131	0.118	0.068	—
n	119	101	122	89	73	11
			α-Glycerol-3-phosphate dehydrogenase			
138	—	—	—	0.009	—	—
100	**0.976**	**1.000**	**0.986**	**0.991**	**0.985**	**1.000**
81	0.024	—	0.014	—	0.007	—
70	—	—	—	—	0.007	—
n	106	61	108	58	67	11

[1] R_f, relative migration; *n*, sample size; S, strong; W, weak.

same gene. The F_{IT} measures the probability of common descent of 2 genes relative to the total population. The fact that the observed value for average F_{ST} is small suggests only a low level of interpopulation divergence. Hartl (1980: 164) gave ranges of values of F_{ST} commonly associated with different levels of genetic divergence. The range of values between 0.05 and 0.15 are considered to represent moderate differentiation between species. The largest values observed for loci sampled in the present study were about 0.03, below the low end of the range. Although these low values do not necessarily mean that divergence between populations in northeastern Arkansas is negligible, they do not suggest the level of genetic divergence commonly observed between races or species.

The same lack of genetic divergence is exhibited by the Nei distance statistics. Within the *An. quadrimaculatus* complex, Nei D values for within-species comparisons generally are less than 0.062, whereas those for interspecific comparisons are greater than 0.105. The smallest distances have been observed between *An. quadrimaculatus* and *An. smaragdinus* (0.092 [Lanzaro et al. 1990], and 0.184 and 0.256 [Narang et al. 1989b, 1989c]) and between *An. deluvialis* and *An. inundatus* (0.128 [Narang et al. 1990]). All other pair-wise comparisons between species yielded distance values greater than 0.51 (Narang et al. 1989b, 1989c). The values for the Nei D statistic observed in comparisons of *An. quadrimaculatus* from northeastern Arkansas were all less than 0.010, offering no evidence for genetic divergence among the populations.

Tests to demonstrate an association between genetic divergence of populations and either geographic distance between localities or different habitat types were inconclusive. The results of the Mantel tests for correlation between geographic and genetic distance matrices were not significantly different from random associations. As an example, Craighead County has its lowest pair-wise F_{ST} value when compared to Jackson County, the site farthest from it, and its largest F_{ST} value with Greene County, which is its closest neighbor. Phenograms produced from either Nei distance statistics or F_{ST} values exhibited no association of genetic divergence with habitat similarities. Again as an example, Craighead County, located on Crowley's Ridge, was clustered with Jackson County, an open rice field habitat, rather than Greene County, the other Crowley's Ridge habitat. In both tests, Jackson County, an open rice field area, exhibited little genetic difference from Mississippi County, the site most distant from it and a swamp habitat located on the opposite side of Crowley's Ridge.

Anopheles quadrimaculatus overwinters as adults. In northeastern Arkansas, these mosquitoes emerge from tree holes, culverts, under bridges, and in and around buildings to feed beginning in June. Populations do not increase substantially until July, after the rice fields are flooded and irrigation is begun on other crops. Anecdotal information and personal observation suggest that only in July do these mosquitoes become a major nuisance in urban areas and on the slopes of Crowley's Ridge. This mosquito has been observed at resting and feeding sites up to 2.4 km from possible oviposition sites (Reinert et al. 1997), suggesting the potential for good dispersal from oviposition centers. It might be expected that any small differences in genetic composition of local, overwintering populations occurring in this region would rapidly be homogenized by gene flow resulting from migration from rapidly increasing populations of the irrigated farmlands during the summer. Alternatively, by waiting to sample populations until after the July population increase, this experiment may have missed real, persistent interpopulation differences that are supported by genetic isolation between the small, local population and the larger migrant population and measured only the genetic homogeneity of the transient migrant population that predominates at these sites during the summer. These alternative possibilities are the subjects of ongoing investigations.

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REFERENCES CITED

Bearden SL. 1996. Genetic variation in populations of *Anopheles quadrimaculatus* and *Psorophora columbiae* in northeastern Arkansas. M.Sc. thesis. Arkansas State University, State University, AR.

Black WC IV, Krafsur ES. 1985a. A FORTRAN program for analysis of genotypic frequencies and description of breeding structure of populations. *Theor Appl Genet* 70: 484–490.

Black WC IV, Krafsur ES. 1985b. A FORTRAN program for calculation and analysis of two-locus linkage disequilibrium coefficients. *Theor Appl Genet* 70:491–496.

Clayton JW, Tretiak DN. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J Fish Res Board Can* 29:1169–1172.

Hartl DL. 1980. *Principles of population genetics* Sunderland, MA: Sinauer Associates, Inc.

Lanzaro GC. 1986. Use of enzyme polymorphism and hybridization crosses to identify sibling species of the mosquito, *Anopheles quadrimaculatus* (Say). Ph.D. dissertation. University of Florida, Gainesville, FL.

Lanzaro GC, Narang SK, Seawright JA. 1990. Speciation in an anopheline (Diptera: Culicidae) mosquito: enzyme polymorphism and the genetic structure of populations. *Ann Entomol Soc Am* 83:578–585.

Narang SK, Kaiser PE, Seawright JA. 1989a. Dichotomous electrophoretic taxonomic key for identification of sibling species A, B, and C of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). *J Med Entomol* 26:94–99.

Narang SK, Kaiser PE, Seawright JA. 1989b. Identification of species D, a member of the *Anopheles quadrimaculatus* species complex: a biochemical key. *J Am Mosq Control Assoc* 5:317–324.

Narang SK, Seawright JA, Kaiser PE. 1990. Evidence for microgeographic genetic subdivision of *Anopheles quadrimaculatus* species C. *J Am Mosq Control Assoc* 6:179–187.

Narang SK, Toniolo SR, Seawright JA, Kaiser PE. 1989c. Genetic differentiation among sibling species A, B, and C of the *Anopheles quadrimaculatus* complex (Diptera: Culicidae). *Ann Entomol Soc Am* 82:508–515.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.

Reinert JF, Kaiser PE, Seawright JA. 1997. Analysis of the *Anopheles* (*Anopheles*) *quadrimaculatus* complex of sibling species (Diptera: Culicidae) using morphological, cytological, molecular, genetic, biochemical, and ecological techniques in an integrated approach. *J Am Mosq Control Assoc* 13(Suppl):1–102.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.

Rutledge CR, Meek CL. 1998. Distribution of the *Anopheles quadrimaculatus* sibling species complex in Louisiana. *Southwest Entomol* 23:161–167.

Seawright JA, Kaiser PE, Narang SK, Tennessen KL, Mitchell SE. 1992. Distribution of sibling species A, B, C, and D of the *Anopheles quadrimaculatus* complex. *J Agric Entomol* 9:289–300.

Sokal RR, Rohlf FJ. 1995. *Biometry* 3rd ed. New York: W.H. Freeman & Co.

Steiner WWM, Joslyn DJ. 1979. Electrophoretic techniques for the genetic study of mosquitoes. *Mosq News* 39:35–54.

Swofford D, Selander RB. 1981. BIOSYS-1; a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J Hered* 72:281–283.

Wright S. 1978. *Evolution and the genetics of populations* Chicago, IL: Univ. Chicago Press.