Population Genetic Structure of Anopheles arabiensis Mosquitoes in Ethiopia and Eritrea


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Abstract

This study examined the population genetic structure of the major malaria vector, Anopheles arabiensis mosquitoes, in Ethiopia and Eritrea. Ethiopia and Eritrea have great geographical diversity, with high mountains, rugged plateaus, deep gorges, and rolling plains. The plateau is bisected diagonally by the Great Rift Valley into the Northwestern Highlands and the Southeastern Highlands. Five A. arabiensis populations from the Northwestern Highlands region and two populations from high-altitude sites in the Great Rift Valley were genotyped using six microsatellite markers to estimate the genetic diversity and population genetic structure of A. arabiensis. We found that A. arabiensis populations from the Northwestern Highlands and the Great Rift Valley region showed a similar level of genetic diversity. The genetic differentiation (F\textsubscript{ST}) of the five mosquito populations within the Northwestern Highlands region was 0.038 (\(P < 0.001\)), while the two populations within the Great Rift Valley showed little genetic differentiation (F\textsubscript{ST} = 0.007, \(P > 0.01\)). The degree of genetic differentiation between the Northwestern Highlands region and the Great Rift Valley region was small but statistically significant (F\textsubscript{ST} = 0.017, \(P < 0.001\)). The population genetic structure of A. arabiensis in the study area did not follow the isolation-by-distance model (\(r^2 = 0.014, P > 0.05\)). The low F\textsubscript{ST} estimates for A. arabiensis populations in Ethiopia and Eritrea are consistent with the general population genetic structure of this species in East Africa based on other molecular markers.

Malaria is a major public health problem in Africa. The World Health Organization (WHO) estimates that there are about 300–500 million clinical malaria cases annually and that 1–1.5 million people die of malaria each year. Anopheles arabiensis and Anopheles gambiae are the principal vectors of malaria in sub-Saharan Africa, but in some areas, such as the Great Rift Valley in East Africa, A. arabiensis is the predominant malaria vector species (Minakawa et al. 2002). A. arabiensis is better adapted to dry environments than A. gambiae (Lindsay et al. 1998). Currently the most efficient malaria prevention method is to reduce human vector contacts using insecticide-impregnated bednets (Goodman et al. 2001; WHO 1993). The mosquito vectors have been developing resistance to insecticides in areas where insecticide-impregnated bednets and pesticide sprays targeting agricultural pests are intensively applied (Chandre et al. 1999; Curtis et al. 1998). Information on the population genetic structure of the anopheline vectors is useful for predicting the spread of insecticide-resistance genes. In addition, this information is critical for designing rational strategies for spreading parasite-inhibiting genes through releasing transgenic mosquitoes to reduce malaria transmission, an approach currently under active investigation (Atkinson and Michel 2002; Crampton et al. 1994).

Ethiopia and Eritrea are located in the horn of Africa. About 75% of the two countries is malarious, with 65% of the population at risk of infection (WHO 1998). The major malaria vector is A. arabiensis; Anopheles funestus and Anopheles nili play a secondary role (Abose et al. 1998). Cytological analyses of A. arabiensis established that 2Rb and 3Ra inversions occur in high frequencies within the Great Rift Valley and in the southern part of Ethiopia (Mekuria et al. 1982, Nigatu et al. 1994). Even though some degree of insecticide resistance has been encountered in different localities, insecticide spray and insecticide-impregnated bednets are the main vector control method (WHO 1998).

Different genetic markers have been used to study the
population genetic structure of *A. gambiae* and *A. arabiensis*, including chromosomal inversions, allozymes, random amplified polymorphic DNA (RAPD), mitochondrial DNA (mtDNA) sequences, and microsatellite markers (Coluzzi et al. 1979, 1985; Lanzaro et al. 1995, 1998; Lehmann et al. 1996b, 1997, 2003; Toure et al. 1998). Microsatellites are tandem repeats of two to five nucleotides and have been regarded as excellent molecular markers for studying population genetic structure because they are very abundant throughout the genome, codominantly inherited, usually neutral, and relatively easy to score at low cost (Bowcock et al. 1994; Estoup et al. 1995; Goldstein and Schlotterer 1999). Previous studies suggest that *A. arabiensis* populations generally show a lower level of genetic differentiation than *A. gambiae*. For example, significant genetic differentiation ($F_{ST} = 0.072–0.100$) was found for *A. gambiae* populations between western Kenya and coastal Kenya using microsatellite markers; hence it was inferred that the Great Rift Valley is a major gene flow barrier for *A. gambiae* (Lehmann et al. 1999, 2003), but no significant genetic differentiation was detected for *A. arabiensis* populations from the two regions using the same loci (Kamau et al. 1999). Similarly Donnelly and Townson (2000) did not detect significant genetic structure for *A. arabiensis* populations within Malawi and Sudan; however, a high level of genetic differentiation was found for *A. arabiensis* populations from two islands that are 240 km apart in the Indian Ocean ($F_{ST} = 0.080–0.215$) (Simard et al. 1999).

In this study we examined the population genetic structure of *A. arabiensis* from Ethiopia and Eritrea, a region that has not been well studied. Ethiopia and Eritrea have great geographical diversity, with high mountains, rugged plateaus, deep gorges, and rolling plains. The Great Rift Valley extends from the southwest to the northeast, and high mountains are generally in the west and southeast regions of Ethiopia. The Rift Valley region is of lower altitude (−115 to +700 m above sea level) and is dotted by lakes and high mountains. The present study included *A. arabiensis* populations from the Northwestern Highlands of Ethiopia and Eritrea, and the Rift Valley in Ethiopia.

### Materials and Methods

#### Study Area

Ethiopia is characterized by a high, mountainous, central plateau bisected diagonally by the Great Rift Valley into the Northwestern Highlands and the Southeastern Highlands. The plateau is rugged and interspersed with towering mountains and deep chasms. Some high mountains exist in the Great Rift Valley. Ethiopia can be divided into four major zones: the central highlands, the western lowlands (one-third of the country), the northeastern lowlands (hot and humid), and the Denakil lowlands (semidesert). Eritrea is located north of Ethiopia, and the country is divided into three major ecological zones: the western lowlands (700–1500 m above sea level); the highlands, with average elevation of 2000 m above sea level; and the coastal plains (0–300 m above sea level), which experience scanty rainfall. The distribution of humans and anopheline mosquitoes is not continuous across this region, but generally clustered on the high-elevation areas where rainfall is abundant.

Mosquitoes were collected in four localities (Arba Minch, Debre Zeit, Gambela, and Inda Sellase) in Ethiopia and three localities (Dasse, Hiletssi, and Adibosqual) in Eritrea (Figure 1). Gambela, Inda Sellase, Dasse, Hiletssi, and Adibosqual are in the Northwestern Highlands region, while Arba Minch and Debre Zeit are located in the Great Rift Valley region. The altitude and the annual average maximum and minimum temperatures and rainfall of each collection locality are listed in Table 1. Mosquitoes were collected using the indoor pyrethrum spray catch method (WHO 1975). Samples from the sites in Eritrea were collected from October to November 1999, and Ethiopian samples were collected from September to October 2001. *A. gambiae* sensu lato were morphologically separated from other anopheline species (*A. pharoensis, A. nili, A. funestus*, and *A. christyi*) and culicine mosquitoes, preserved in 95% ethanol, and kept at $−20^\circ$C.

#### DNA Extraction and Species Identification

Genomic DNA was extracted from individual female mosquitoes according to Yan et al. (1997). Because *A. gambiae* and *A. arabiensis* cannot be distinguished by morphology, the rDNA–polymerase chain reaction (PCR) method was used to determine the species identity for specimens within the *A. gambiae* species complex (Scott et al. 1993). If a specimen failed in the initial PCR amplification, the specimen was tested in the second or third PCR until a success-
ful amplification was achieved. An individual was scored as unknown if it could not be identified by three PCR tests.

Microsatellite Loci and Genotype Scoring

Six microsatellite markers developed for *A. gambiae* were used to genotype *A. arabiensis*. Other anopheline species were not studied because of insufficient samples. These microsatellite markers, including *AGXH1D1* and *AGXH131* on chromosome X, *AG2H46* and *AG2H79* on chromosome 2, and *AG3H29C* and *AG3H33C* on chromosome 3, can be readily amplified using *A. arabiensis* genomic DNA as a template (Lehmann et al. 1996b; Zheng et al. 1996). The cytological locations of the microsatellite markers in relation to *A. arabiensis* chromosomal inversions are unknown. Microsatellite analyses were conducted on 39–60 individuals per population (Table 1).

A Li-Cor Model 4200 Automated DNA Analyzer (Li-Cor Inc., Lincoln, NE) was used for gel electrophoresis. For the apparatus to detect PCR products, one primer in every pair of microsatellite primers must be fluorescently labeled. To reduce the cost associated with synthesis of fluorescently labeled primers, we used the “tailed primer” method (Oetting et al. 1998; Sharakhov et al. 2001); that is, the forward primer for each microsatellite locus was synthesized with an additional 19 bp sequence (5’GACGACGTTGTAAAACGAC3’) added to the 5’end of the primer. A third primer with the same 19 bp sequence was used to label the forward primer. A third primer with the same 19 bp sequence was used for the detection of all microsatellite alleles. The tailed primer method reduced the cost of oligonucleotide synthesis by more than 80%. The 10 μl PCR reaction contained 1X Taq buffer, 0.2 mM dNTPs, 1.5 pmol forward and reverse primers, 1.5 pmol fluorescently labeled 19 bp sequences, 1.5 mM MgCl₂, 1.0 μg BSA, 1.0 unit Taq polymerase, and about 20 ng genomic DNA. Cycling conditions in an MJ Research PTC-220 thermocycler were 35–40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. Allele sizes were determined using Gene ImagIR computer software (Scanalytics Inc. 1998). The allele sizes used in the analysis were true allele sizes that were adjusted for the 19 bp tail in the forward primer.

### Table 1. Description of mosquito sampling sites in Ethiopia and Eritrea

<table>
<thead>
<tr>
<th>Country</th>
<th>Locality</th>
<th>Altitude (m)</th>
<th>Annual mean of maximum/minimum temperature (°C)</th>
<th>Annual rainfall (mm)</th>
<th>Number of houses</th>
<th>Number of mosquitoes analyzed</th>
<th>Collection period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Debre Zeit</td>
<td>1850</td>
<td>18/6</td>
<td>905</td>
<td>23</td>
<td>60</td>
<td>Sept.–Oct. 2001</td>
</tr>
<tr>
<td></td>
<td>Gambalet</td>
<td>539</td>
<td>34/19</td>
<td>1107</td>
<td>15</td>
<td>60</td>
<td>Sept.–Oct. 2001</td>
</tr>
<tr>
<td></td>
<td>Inda Sellas</td>
<td>2182</td>
<td>25/7</td>
<td>507</td>
<td>17</td>
<td>60</td>
<td>Sept.–Oct. 2001</td>
</tr>
<tr>
<td>Eritrea</td>
<td>Dasse</td>
<td>680</td>
<td>35/20</td>
<td>600</td>
<td>10</td>
<td>36</td>
<td>Oct.–Nov. 1999</td>
</tr>
<tr>
<td></td>
<td>Hiletsidi</td>
<td>450</td>
<td>36/22</td>
<td>500</td>
<td>10</td>
<td>45</td>
<td>Oct.–Nov. 1999</td>
</tr>
<tr>
<td></td>
<td>Adibosqual</td>
<td>1100</td>
<td>28/15.5</td>
<td>550</td>
<td>10</td>
<td>50</td>
<td>Oct.–Nov. 1999</td>
</tr>
</tbody>
</table>

### Data Analysis

Microsatellite polymorphism was measured by the number of alleles and heterozygosity at each locus. Using the probability test in the GENEPOP computer program (Raymond and Rousset 1995), conformance with Hardy-Weinberg equilibrium (HWE) was tested for each locus and population and the Bonferroni correction was applied for multiple comparisons. We further determined whether distortion from HWE resulted from deficient or excessive heterozygosity using the *F*ₜₛ statistics and probability test. Because the probability test is robust to low allele frequencies, rare alleles were not pooled. Variations in heterozygosity among the populations were analyzed following the method of Weir (1990), using the analysis of variance (ANOVA) with subpopulations, individuals, loci and interactions of loci, and individuals as factors. All factors were treated as random effects except loci. The Fisher exact test was performed to detect genotypic linkage disequilibrium for pairs of loci in each population (Raymond and Rousset 1995). Population genetic structure was examined using Wright’s *F*-statistics (*F*ₜₛ) using the FSTAT computer program (Goudet 1995). The standard deviations (SD) of the *F*-statistics were obtained for each locus by a jackknife procedure over all the alleles. These were used to test the statistical significance. *R*ₜₛ statistics, analogous to *F*ₜₛ but specifically for microsatellite markers (Slatkin 1995), were not calculated because *R*ₜₛ is particularly sensitive to the assumptions underlying microsatellite evolution (Gaggiotti et al. 1999), but the mode of microsatellite evolution in anopheline mosquitoes is unknown. In addition, the microsatellite markers used in this study were isolated from *A. gambiae*, converting microsatellite allele size to the number of tandem repeats based on *A. gambiae* genome sequence is not appropriate. Genetic differentiation between regions (e.g., Ethiopian versus Eritrean populations, and Northwestern Highlands and the Great Rift Valley populations) was calculated by nested ANOVA.

The isolation-by-distance model of population genetic structure was tested by linear regression of pairwise *F*ₜₛ/ (1 − *F*ₜₛ) against the natural logarithm of straight-line geographical distance between population pairs (Rousset 1997). The statistical significance of the regression was tested using the Mantel test with 2000 permutations. The pairwise geographical distance of the sampling sites ranged from 76 to 877 km.
Table 2. Number of alleles (n), observed heterozygosity (H<sub>o</sub>), and inbreeding coefficients (F<sub>IS</sub>) of microsatellite markers in seven A. arabiensis populations from Ethiopia and Eritrea

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Locus</th>
<th>Arba Minch</th>
<th>Debre Zeit</th>
<th>Gambela</th>
<th>Inda Sellase</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>AGXH1D1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>AGXH131</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>AG2H46</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>AG2H79</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>AGH29C</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>AGH33C</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>4.5</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Results

Species Identification

A total of 550 mosquitoes in the A. gambiae species complex, collected from seven localities in Ethiopia and Eritrea, were identified as A. arabiensis in the PCR identifications. Neither A. gambiae nor A. quadriannulatus were detected.

Genetic Diversity, HWE, and Linkage Disequilibrium

Population genetic diversity can be measured by the number of alleles and expected heterozygosity. Loci AGXH1D1 and AGH329C were the least polymorphic markers, while loci AG2H46 and AGH33C were the most polymorphic (Table 2). The average number of alleles over the six loci ranged from 4.5 to 5.2 per locus and did not vary significantly among the populations (F = 0.05, df = 6, P > .05). Among the seven studied populations, the expected heterozygosity ranged from 0.158 to 0.274 per locus. The observed heterozygosity over the six loci did not differ significantly (F = 0.53, df = 6, P > .05). The genotype frequencies at several loci did not conform to HWE. Of the 42 tests (6 loci × 7 populations) for conformance to HWE at the locus level within populations, 13 tests (31%) showed significant distortion from HWE, and the majority (11 tests) were due to significant heterozygote deficiency (Table 2). Locus AG2H46 showed significant heterozygote deficiency in three of seven populations, likely resulting from the presence of null alleles (Lehmann et al. 1996a). The maximum likelihood estimate of null allele frequency is 0.063, 0.229, and 0.363 for Debre Zeit, Dasse, and Hiletsidi, respectively. Exact tests revealed that only four pairs of loci out of a total of 105 possible pairs (3.8%) were in linkage disequilibrium (data not shown), suggesting a very low level of linkage disequilibrium among the six loci studied.

Population Genetic Structure and Isolation by Distance

The seven populations exhibited remarkably similar allele distributions and shared the same predominant alleles at four loci (AGXH1D1, AGXH131, AGH79, and AGH29C; data not shown; microsatellite allele frequency data are available to interested parties upon request). The genetic differentiation of the four northern populations (Adibosqual, Hiletsidi, Dasse, and Inda Sellase) (F<sub>ST</sub> = 0.046, P < .001) was sixfold higher than the three southern populations (Gambela, Debre Zeit, and Arba Minch) (F<sub>ST</sub> = 0.007, P < .01; Table 3), despite the fact that the four northern sites are more tightly clustered. The genetic differentiation (F<sub>ST</sub>) between populations from the northern and southern regions was 0.018 (P < .05). Very small genetic differentiation existed for the two populations in the Great Rift Valley (Debre Zeit and Arba Minch) (F<sub>ST</sub> = 0.007, P < .01; Table 3), and between the Northwestern Highlands and the Great Rift Valley regions (F<sub>ST</sub> = 0.017, P < .001).

A Mantel test revealed no significant correlation between pairwise F<sub>ST</sub>/ (1 - F<sub>ST</sub>) against the natural logarithm of pairwise geographical distance (r = 0.014, P = .15), suggesting that the population genetic structure of A.
Genetic diversity of the seven *A. arabiensis* populations used in this study was similar, as measured by the number of alleles and observed heterozygosity. Because our study and others used several common microsatellite markers, it is possible to compare these results. Our estimate of genetic diversity was similar to the Kenyan population (Kamau et al. 1999), but considerably lower than the populations in other East African countries, for reasons that are unclear. For example, the observed heterozygosity of *A. arabiensis* populations in nine localities along a 4500 km transect from Sudan to Mozambique ranged from 0.47 to 0.73 for locus AG3H33C and from 0.527 to 0.732 for AG2H46 (Donnelly and Townson 2000). In comparison to one Ethiopian population used by Donnelly and Townson (2000), our current study with comparable sample sizes detected one to two fewer alleles and 30% less observed heterozygosity in these two loci. That fewer alleles were detected in our study was not likely caused by noncanonical alleles that have either gone undetected or been binned into other classes, because deviation from HWE was observed in only 5 of 42 tests (12%). Further, the heterozygosity deficit occurred only in locus AG2H46, a locus where null alleles are known to occur in *A. gambiae* (Lehmann et al. 1996a).

We observed small, but statistically significant genetic structure for *A. arabiensis* populations in Ethiopia and Eritrea (*F_{ST} = 0.034*), and between the Northwestern Highlands and the Great Rift Valley regions (*F_{ST} = 0.017*). The extent of genetic differentiation between the Northwestern Highlands and the Great Rift Valley regions was about half of the within-population variation of the Northwestern Highlands region (*F_{ST} = 0.038*). The particular geographic location of these two sites in the Great Rift Valley may cause this phenomenon: These two sites are located in the extension of the mountainous plateau of the Northwestern Highlands area, as indicated by the high elevation of the sites, and thus there is no obvious physical barrier between these two particular study sites in the Great Rift Valley and the Northwestern Highlands. The geographic distance between the northern and southern populations should not be a major factor because the population genetic structure of *A. arabiensis* in the study area did not follow the isolation-by-distance model.

**Discussion**

This study confirmed that *A. arabiensis* is the predominant malaria vector species in Ethiopia and Eritrea. *A. arabiensis* populations from the Northwestern Highlands region and the Great Rift Valley region had similar levels of genetic diversity as measured by the number of alleles and observed heterozygosity. Small, but statistically significant genetic structure was identified between Ethiopian and Eritrean populations, and between the Northwestern Highlands and the Great Rift Valley regions. The population genetic structure of *A. arabiensis* in Ethiopia and Eritrea did not conform to the isolation-by-distance model.

In our mosquito sampling from Ethiopia and Eritrea, *A. pharoensis*, *A. nili*, *A. funestus*, and *A. christyi* were also found, but *A. gambiae* and *A. quadrimaculatus* were not detected. This result is consistent with previous findings that *A. arabiensis* is the predominant vector responsible for malaria transmission in Ethiopia (Abose et al. 1998; Donnelly et al. 2001; Lulu et al. 1991; Mekuria et al. 1982; Nigatu et al. 1994). Climatic factors, such as precipitation and temperature, are important determinants of the range and relative abundance of individual species of the *A. gambiae* complex (Lindsay et al. 1998). *A. gambiae* is usually the predominant species in saturated environments, but *A. arabiensis* is more common in arid areas. Although climatic conditions are directly associated with elevation (temperature decreases and rainfall generally increases with elevation), elevation is not a good predictor of malaria vector species distribution. For example, in western Kenya, *A. gambiae* but not *A. arabiensis* was found in areas with an elevation greater than 1500 m above sea level, and *A. gambiae* was more abundant than *A. arabiensis* in the basin region of Lake Victoria, which has an elevation of 1200–1300 m above sea level (Minakawa et al. 2002; Shillu et al. 1998). In the present study, no *A. gambiae* was found in sampling sites with an elevation of 450 to 2182 m above sea level in Ethiopia and Eritrea. Moisture index, calculated as the ratio of precipitation to potential evapotranspiration, is a better predictor of *A. gambiae* and *A. arabiensis* species distribution (Lindsay et al. 1998; Minakawa et al. 2002).

A. *pharoensis* in Ethiopia and Eritrea did not conform to the isolation-by-distance model.

### Table 3. *F_{ST}* estimates of *A. arabiensis* populations from Ethiopia and Eritrea

<table>
<thead>
<tr>
<th>Locus</th>
<th>of all 7 populations</th>
<th>of 4 northern populations</th>
<th>of 3 southern populations</th>
<th>between northern and southern regions</th>
<th>of 5 populations in western highlands</th>
<th>of 2 populations in Rift Valley</th>
<th>between western highlands and Rift Valley</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGXH1D1</td>
<td>0.002</td>
<td>0.000</td>
<td>0.001</td>
<td>0.060</td>
<td>0.002</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>AGXH131</td>
<td>0.011*</td>
<td>0.021*</td>
<td>0.004</td>
<td>0.001</td>
<td>0.013**</td>
<td>0.008</td>
<td>0.002</td>
</tr>
<tr>
<td>AG2H46</td>
<td>0.042***</td>
<td>0.060***</td>
<td>0.016**</td>
<td>0.018*</td>
<td>0.048***</td>
<td>0.021**</td>
<td>0.017***</td>
</tr>
<tr>
<td>AG2H79</td>
<td>0.019**</td>
<td>0.027**</td>
<td>0.003</td>
<td>0.009*</td>
<td>0.018**</td>
<td>0.004</td>
<td>0.017*</td>
</tr>
<tr>
<td>AG3H29C</td>
<td>0.152***</td>
<td>0.142***</td>
<td>0.001</td>
<td>0.101***</td>
<td>0.145***</td>
<td>0.001</td>
<td>0.073***</td>
</tr>
<tr>
<td>AG3H33C</td>
<td>0.007**</td>
<td>0.012</td>
<td>0.004**</td>
<td>0.001</td>
<td>0.008**</td>
<td>0.000</td>
<td>0.004</td>
</tr>
<tr>
<td>Overall</td>
<td>0.034***</td>
<td>0.046***</td>
<td>0.007**</td>
<td>0.018*</td>
<td>0.038***</td>
<td>0.007**</td>
<td>0.017***</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, *** P < 0.001.
this study are consistent with the general population genetic structure pattern of *A. arabiensis* based on microsatellite and other molecular markers. Donnelly and Townson (2000) reported an $F_{ST}$ value of 0.035 for nine *A. arabiensis* populations spanning 4500 km from north Sudan to south Mozambique and found that the population genetic structure is consistent with the isolation-by-distance model. Kamau et al. (1999) showed no significant genetic structure of *A. arabiensis* populations between western and coastal Kenya that are separated by 700 km distance and the Great Rift Valley. Besansky et al. (1997) reported that the *A. arabiensis* populations from east Africa (Kenya), south Africa, and west Africa (Senegal) had an $F_{ST}$ of 0.038 by allozyme and 0.044 by mtDNA sequence variation. The largest $F_{ST}$ (0.080–0.215) reported in the literature was a comparison of *A. arabiensis* populations from Senegal and the east outer islands (Madagascar, and Reunion and Mauritius Islands) (Simard et al. 1999). The authors proposed that historical forces of drift rather than mutation were probably the forces generating genetic divergence between these populations, because homogenization of the gene pool by migration is restricted by the ocean.

Two hypotheses can explain the low genetic differentiation of *A. arabiensis* populations across the African continent. The first is that there may not be significant gene flow barriers for *A. arabiensis* because it is widespread in Africa. The second is that *A. arabiensis* populations in Africa resulted from recent expansions and the large effective population size (Donnelly et al. 1999; Lehmann et al. 1996b; Taylor et al. 1993). Even if rugged mountains and deep chasms in our study area are indeed a significant gene flow barrier for the mosquitoes, the effect on the population genetic structure may not be apparent because genetic heterogeneity may be caused by historical factors, such as population expansion, rather than by current migration. Thus gene flow inferred from the $F_{ST}$ statistics should be interpreted with caution.

**Acknowledgments**

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**References**


