

DISTRIBUTION OF ANOPHELINE MOSQUITOES IN ERITREA

JOSEPHAT SHILILU, TEWOLDE GHEBREMESKEL, SOLOMON MENGISTU, HELEN FEKADU, MEHARI ZEROM,
CHARLES MBOGO, JOHN GITHURE, WEIDONG GU, ROBERT NOVAK, AND JOHN C. BEIER

National Malaria Control Program, Division of Communicable Diseases Control, Ministry of Health, Asmara, Eritrea; International Center of Insect Physiology and Ecology, Nairobi, Kenya; Centre for Geographic Medicine Research, Kenya Medical Research Institute, Kilifi, Kenya; Illinois Natural History Survey, Champaign, Illinois; Department of Epidemiology and Public Health, University of Miami School of Medicine, Miami, Florida

Abstract. The spatial distribution of anopheline mosquito species was studied throughout Eritrea during the 1999–2001 malaria transmission seasons from October to December for the highlands and western lowlands and February to April for the coastal region. Of the 302 villages sampled, 59 were visited in both the first and second year. Overall, 13 anopheline species were identified, with the *Anopheles gambiae* complex predominating during the first year (75.6%, $n = 861$) and the second year (91.9%, $n = 1,262$). Intrazonal variation accounted for 90% of the total variation in mosquito distribution. Polymerase chain reaction results indicated that 99% ($n = 1,309$) of the *An. gambiae* s.l. specimens were *An. arabiensis*, indicating that this was the only member of the *gambiae* complex present. There was a high degree of aggregation of anophelines within zones and villages, with more than 80% of the total anophelines being collected from less than 20% of the villages and from only 10% of the houses sampled. At least 80% of the anopheline mosquitoes were collected from grass-thatched Agudo-type housing. Vector abundance showed an inverse relationship with elevation, with highest densities in the low-lying western lowlands. Multiple regression analysis of log-transformed mean density of *An. arabiensis* with rainfall and the normalized difference vegetation index (NDVI) (average NDVI, minimum NDVI, and maximum NDVI) showed that these independent variables were not significantly associated with mosquito densities ($R^2 = 0.058$). Our study contributes to the basic understanding of the ecology and distribution of malaria vectors with respect to species composition and spatial heterogeneities both that could be used to guide vector control operations in Eritrea.

INTRODUCTION

Malaria accounts for more than 30% of the total outpatient morbidity in Eritrea and about 28% of all hospital admissions (Ministry of Health, unpublished data). The country is divided into three main epidemiologic strata. The first is the western lowlands at 500–1,500 meters above sea level. In this region, malaria is highly seasonal and the area is prone to epidemics. Transmission is perennial along rivers, valleys, and in irrigation projects. The second is the coastal plain along the Red Sea that experiences scanty rainfall and has generally low malaria endemicity. The third is the highlands with an average elevation of 2,000 meters above sea level that experiences generally very low levels of malaria. Almost 67% of the residents of Eritrea live in malaria-endemic areas.

Although malaria remains a major cause of mortality in Eritrea, especially among children less than five years of age, little is known about the *Anopheles* mosquito species responsible for transmission. The only recent data on vector species comes from studies conducted at five sites in western Eritrea along the Eritrea–Ethiopia border.¹ These studies focused only on the *Anopheles gambiae* complex and were limited in geographic range. Some information on vector bionomics could be extrapolated from studies done in neighboring Ethiopia, but this would not describe the distribution of vectors in Eritrea due to ecologic differences between the two countries.^{2,3} Ecologic diversity plays an important role in determining how mosquito populations are structured since each mosquito species has a geographic range that is limited according to its physiologic tolerances to existing environmental conditions.

Early studies indicated that 34 *Anopheles* species were present in Ethiopia and that up to 19 species were present in Eritrea.⁴ The spatial patterns of these anopheline species would provide insight into the dynamics of malaria parasite transmission that are needed to guide vector control operations. The success of vector control largely depends on an

understanding of the bionomics of the anopheline species responsible for malaria transmission.⁵ The present paper describes the first detailed study on the species composition and spatial distribution of *Anopheles* mosquitoes in Eritrea. These data are necessary for developing efficient and focused vector control strategies.

MATERIALS AND METHODS

Country profile. Eritrea is situated in the horn of Africa and lies between longitudes 36°30'E and 43°20'E and latitudes 12°42'N and 18°2'N. It is bordered by Sudan to the north and northwest, Ethiopia to the south, Djibouti on the southeast, and the Red Sea to the east (Figure 1). It has an area of approximately 124,000 km², including the Dahlak Archipelago and the islands in the Red Sea. Rainfall is scanty and highly seasonal and ranges from 400 mm to 650 mm per year in the highlands and from 200 mm to 300 mm in the lowlands. Daytime temperatures range from 16°C to more than 40°C in different parts of the country. The rainy season occurs in the central highlands and the western lowlands between July and September and in the coastal plains from October to April. The peak malaria transmission is experienced at the end of the rainy season, and occurs in the coastal zone between February and April and in the central highlands and western lowlands from October to December. The country is divided into six administrative zones and 56 sub-zones containing approximately 1,500 villages. Villages are isolated due to the nucleated nature of settlement and houses within villages are generally close to each other. The estimated population is approximately 3.5 million, with about 10% of the population living in urban areas.

Study sites. Thirty villages were selected in each of five zones, i.e., Anseba, Debub, Maekel, Gash-Barka, and Northern Red Sea (NRS) in the first and second year of the survey, except for the Maekel zone, where only 15 villages were

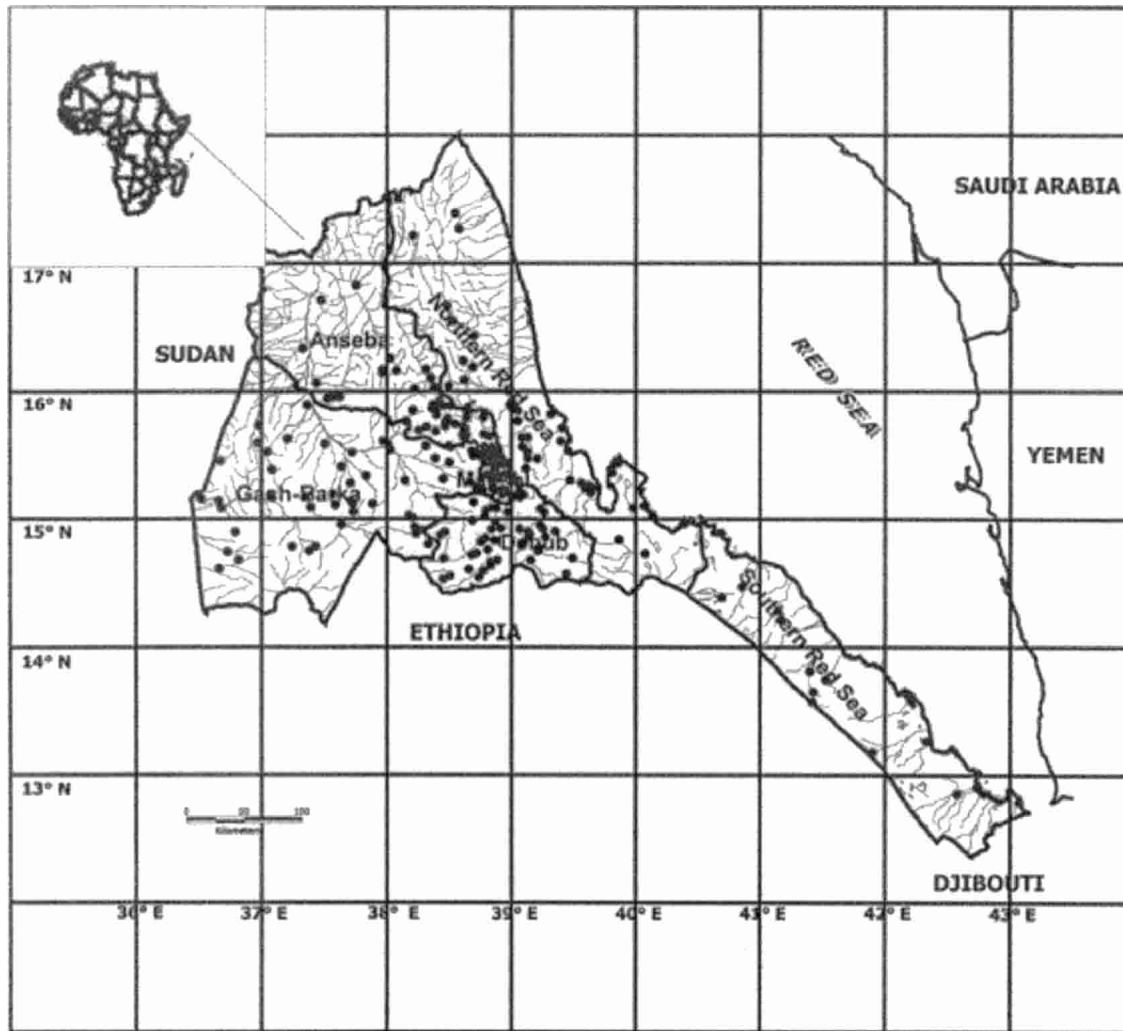


FIGURE 1. Map of Eritrea showing the six administrative zones and the location of the study villages. Eritrea borders Ethiopia, Sudan, and Djibouti in the Horn of Africa, and lies at the northernmost limit of the distribution of malaria in east Africa, with transmission varying from stable to epidemic.

sampled during the second year and for the Southern Red Sea zone (SRS), where studies were conducted only in 17 villages during the first year. A total of 176 and 135 villages were surveyed during the first and second phases of the study, respectively (Figure 2). The selection of villages was based on the sub-zones, with at least two villages randomly selected from each sub-zone. Fifty-nine villages that reported either very high or low vector densities during the first year were sampled again during the second year. In each village, mosquito sampling was conducted in 10 randomly selected houses, 5 at the periphery of the village and 5 at the center. The houses comprised the four main housing types found in the country, i.e., Agudo, rectangular, Hudmo, and portable houses. Agudo houses are grass-thatched with mud walls. Rectangular houses are stonewalled with roofs made of tin. Hudmo houses are distinguished by their curved roofs that are made of wood and compacted soil. The portable house types are temporary, movable structures made of sticks covered with mats. The walls in the houses were made of mud, mats, plaster, stone, thatch, tin, or sticks.

Mosquito sampling and processing. The surveys were conducted over two malaria transmission seasons. In the Anseba,

Debub, Gash-Barka and Maekel zones, sampling was conducted from October to December in 1999 and 2000, respectively, coinciding with the peak malaria transmission season. In the NRS and SRS zones, sampling was conducted between February and April in 2000 and 2001. Sampling of mosquitoes in each village was done only once. In each selected village, indoor resting anopheline mosquitoes were collected inside 10 randomly selected houses by pyrethrum spray catches conducted from 6:00 AM to 9:00 AM. The female anopheline mosquitoes were placed in petri dishes lined with moist cotton wool and filter paper and later identified to species by morphologic criteria.^{6,7}

Polymerase chain reaction (PCR) and determination of sporozoite rate. A proportion of the *An. gambiae s.l* specimens were identified to sibling species by a ribosomal DNA PCR using primers specific for *An. arabiensis*, *An. gambiae*, and *An. quadriannulatus*.⁸ DNA was extracted from wings and legs.⁹ The head and thorax of each mosquito was separated from the abdomen and tested for the presence of *Plasmodium falciparum* circumsporozoite protein.¹⁰ Mosquitoes were ground in 50 μ l of boiled casein containing Nonidet 40, with the final volume brought to 250 μ L with blocking buffer.

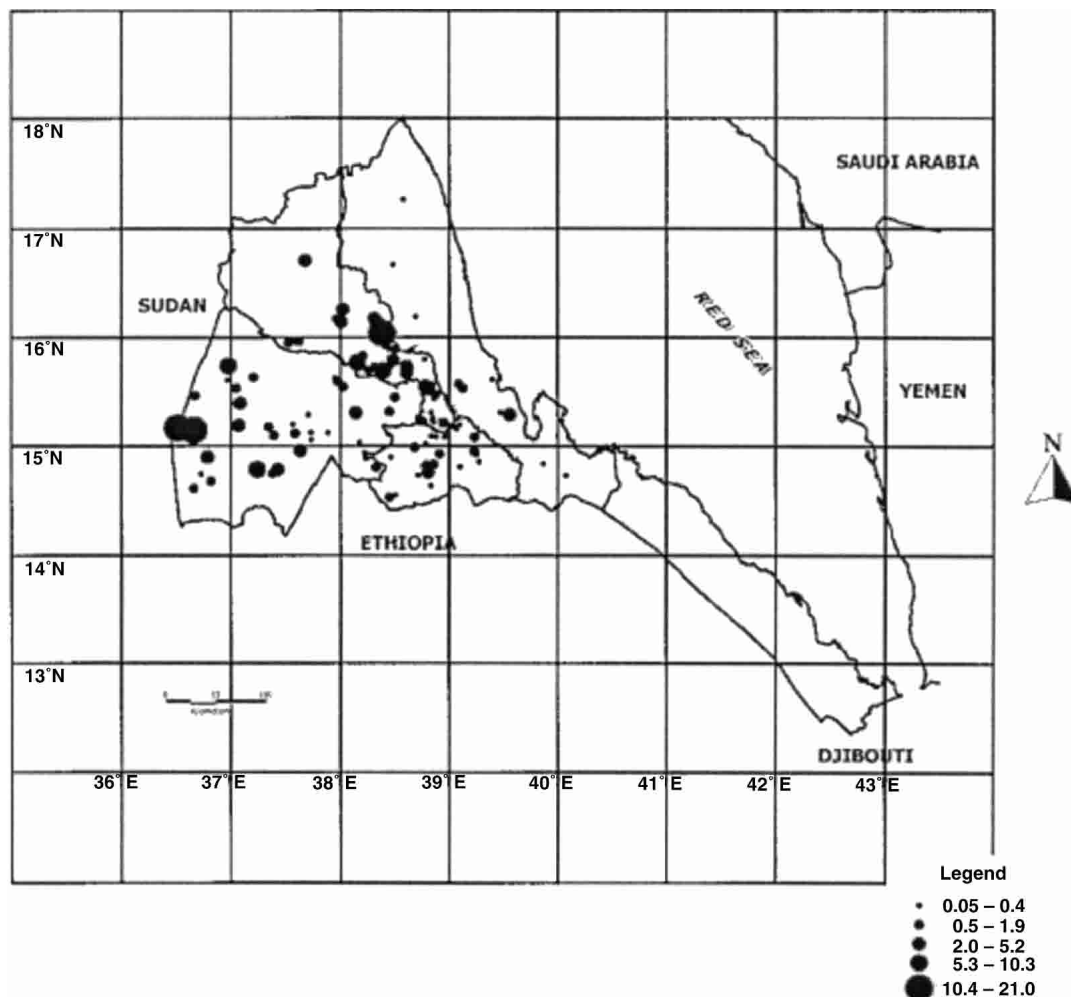


FIGURE 2. Spatial variation in the densities of *Anopheles* mosquitoes in Eritrea. *Anopheles* species are found throughout the country, with a highly aggregated spatial distribution (51.5% in the Gash-Barka zone and 34.5% in the Anseba zone).

Fifty microliters of the triturate was used in an enzyme-linked immunosorbent assay and positive reactions were determined visually.¹⁰

Statistical analysis. Density of anopheline mosquitoes was expressed as the number of female anophelines per house for each species. Log transformation [$\log_{10}(x + 1)$] of the data was done to stabilize the variance. Statistical analyses were performed only for *An. gambiae s.l.*, the predominant species (84.5% of the total specimens). An analysis of variance (ANOVA) examined the effects of zones, elevation, house type, wall type, and their interactions. We grouped houses into three categories of elevation, <500, 500–900, and >900 meters above sea level, as low, medium, and high, respectively. The analyses were performed using a general linear model (GLM) with SPSS version 11.5 software (SPSS Inc., Chicago, IL). Because of missing cells in interaction analyses, type IV (sums of squares) model was specified in the GLM. We excluded samples collected from Hudmo and portable houses because they were sampled in only two zones (Anseba and NRS). Similarly, the samples collected from houses with sticks or tin walls were not included in the analyses because a small number of houses with these types of walls were sampled. Multiple regression analysis examined relationships

between *An. gambiae s.l.* densities and environmental variables. Rainfall data was derived from Government of Eritrea rainfall distribution maps from 1990 to 2000. The rainfall estimates were grouped into five categories (≤ 200 , 201–300, 301–400, 401–500, and >500 mm per year). The normalized difference vegetation index (NDVI) data was derived from the Famine Early Warning System using National Oceanic and Atmospheric Administration satellites.

RESULTS

Vector species composition and abundance. A total of 2,513 adult female anopheline mosquitoes representing 13 species were collected during the two sampling periods (Table 1). *Anopheles gambiae s.l.* (84.5%) was the most abundant species, followed by *An. d'thali* (9.4%) and *An. cinereus* (3.4%). The rest of the 10 species comprised less than 3% of the total catch. The density of *An. gambiae s.l.*, expressed as number of female mosquitoes per house, varied significantly among the four zones (Table 2). The highest density of *An. gambiae s.l.* was found in the areas covering the western escarpments, which comprised a large part of the Anseba zone, and the western lowlands of the Gash-Barka zone (Figure 2).

TABLE 1

Species composition and relative abundance of adult *Anopheles* mosquitoes collected between October 1999 and April 2001 in Eritrea*

Species	Location					Total
	Gash Barka	Anseba	Debut	Maekel	NRS	
<i>An. gambiae s.l.</i>	1,294	606	147	8	69	2,124†
<i>An. d'thali</i>	0	216	2	0	17	235
<i>An. cinereus</i>	0	12	24	48	0	84
<i>An. squamosus</i>	0	0	0	20	0	20
<i>An. rhodesiensis</i>	0	19	1	0	0	20
<i>An. rupicolus</i>	0	11	0	0	1	12
<i>An. harperi</i>	1	0	0	0	0	1
<i>An. demeilloni</i>	0	3	1	0	0	4
<i>An. garnhami</i>	0	3	0	0	0	3
<i>An. funestus</i>	0	0	2	0	0	2
<i>An. chrysti</i>	0	0	0	3	0	3
<i>An. wellcomi</i>	0	0	1	0	3	4
<i>An. pharoensis</i>	0	0	0	1	0	1
Total	1,295	870	178	80	90	2,513

* *An. gambiae s.l.* was the predominant species of the 13 *Anopheles* species sampled. NRS = Northern Red Sea.

† Polymerase chain reaction analysis of *An. gambiae s.l.* specimens indicated that >99% (n = 1,309) were *An. arabiensis*.

Anopheles d'thali was sampled predominantly from the Anseba zone (75%, n = 216), whereas a large proportion of *An. cinereus* (57%, n = 48) was found in the Maekel zone at elevations > 1,800 meters above sea level.

The Debut zone had the highest diversity of anopheline species. Of the 13 *Anopheles* species sampled, 8 were found in the Debut zone, 6 each in the Anseba and Maekel zones, and only 2 species in the Gash-Barka zone. Geographic variation in anopheline species composition is shown in Figure 3. In 59 villages where sampling was conducted in both the first and second years of the study, no significant difference in mean density of *An. gambiae s.l.* was found between years ($t = 0.433$, degrees of freedom = 58, $P = 0.667$). *Anopheles d'thali* was collected more frequently in the first year of the study than in the second year (Table 3).

Analysis of *An. gambiae s.l.* sibling species by PCR. To identify the *An. gambiae s.l.* species present, 1,446 specimens were analyzed by PCR using primers specific for *An. gambiae s.s.*, *An. arabiensis*, and *An. quadriannulatus*. Of the total mos-

TABLE 2

Analysis of variance examining variation in *Anopheles arabiensis* densities in the countrywide survey between October 1999 and April 2001 in Eritrea

Factors	Degree of freedom	F P
Intercept	1	0.088 0.77
Zone	4	5.613 <0.01
Elevation	2	8.303 <0.01
House type	1	0.017 0.895
Wall type	4	2.225 0.064
Zone × elevation	4	4.852 <0.01*
Zone × house type	4	2.545 0.038*
Zone × wall type	13	1.499 0.110
Elevation × house type	2	0.374 0.688
Elevation × wall type	8	4.343 <0.01*
House type × wall type	4	5.086 <0.01*
Error	2,619	

* The analysis of variance model examined the effect of zone, elevation, house type, and wall type on *An. arabiensis* densities and showed significant interactions at four levels ($P < 0.05$).

quitoes identified, more than 99% (n = 1,309) were *An. arabiensis*, indicating that this may be the only representative of the *gambiae* complex present. Specimens of the *gambiae* complex hereafter are referred to as *An. arabiensis*. Only one mosquito was identified as *An. gambiae sensu stricto* and no *An. quadriannulatus* was identified among the *gambiae* complex specimens tested.

Plasmodium falciparum sporozoite rates. Of the 1,505 anopheline mosquitoes tested, only five were positive for *P. falciparum* circumsporozoite antigen, yielding a low sporozoite rate of 0.33%. Of the five positive anophelines, one positive *An. cinereus* was collected in the Maekel zone at an elevation of more than 2,000 meters, one *An. d'thali* was collected in the Anseba zone, and three *An. arabiensis* (0.2%) were collected in the Anseba (n = 1) and Gash Barka (n = 2) zones.

Spatial variation in *Anopheles* densities. Our data showed that there was distinct aggregation of *An. arabiensis* within zones and villages. Overall, more than 80% of this species was sampled from less than 20% of the villages in all the zones. Within villages, 80% of the total *An. arabiensis* collected came from 1.8%, 2.0%, 8.5%, and 10.2% of the houses sampled in the NRS, Debut, Anseba, and Gash Barka zones, respectively. *Anopheles d'thali* and *An. cinereus* showed similar clumped distribution patterns, with more than 90% of the two species collected from only 2% of the 2,850 houses sampled countrywide.

The density of *An. arabiensis* varied significantly with elevation (Table 2), being most abundant between 500 and 900 meters above sea level. This elevation covers the western escarpments, which comprise a large part of the Anseba zone and the western lowlands of the Gash-Barka zone. Low numbers were found in villages more than 900 meters above sea level, although 4.4% (n = 110) of the *An. arabiensis* specimens were collected in villages located more than 1,800 meters above sea level. The ANOVA model used to examine the effects of zones, elevation, house type, and wall type on vector densities showed significant two-way interactions (Table 2). Collections of *An. arabiensis* made at high elevations were similar to those from low elevations. However, in the Gash-Barka zone, collections at high elevations were significantly greater than those from low elevations (Figure 4A). Similarly, the difference in *An. arabiensis* densities between the Agudo and rectangular house types was only significant in the Gash-Barka zone (Figure 4B). Captures of *An. arabiensis* in Agudo houses were generally greater than in rectangular houses except in houses with thatched walls (Figure 4C). Among houses with different wall types, *An. arabiensis* densities were generally lower in stone walled houses at low and medium elevations, but at high elevations, stone walled houses had more *An. arabiensis* (Figure 4D).

We conducted multiple regression analysis of log-transformed mean density of *An. arabiensis* with rainfall and NDVI (average, minimum, and maximum) as independent variables. The low amount of variation ($R^2 = 0.058$) in the dependent variable explained by the combination of the independent variables (rainfall and NDVI summaries) in the regression model indicated that the two variables were not good predictors of *An. arabiensis* density (Table 4). Rainfall was the only significant variable in the model, though independently, it had low explanatory power.

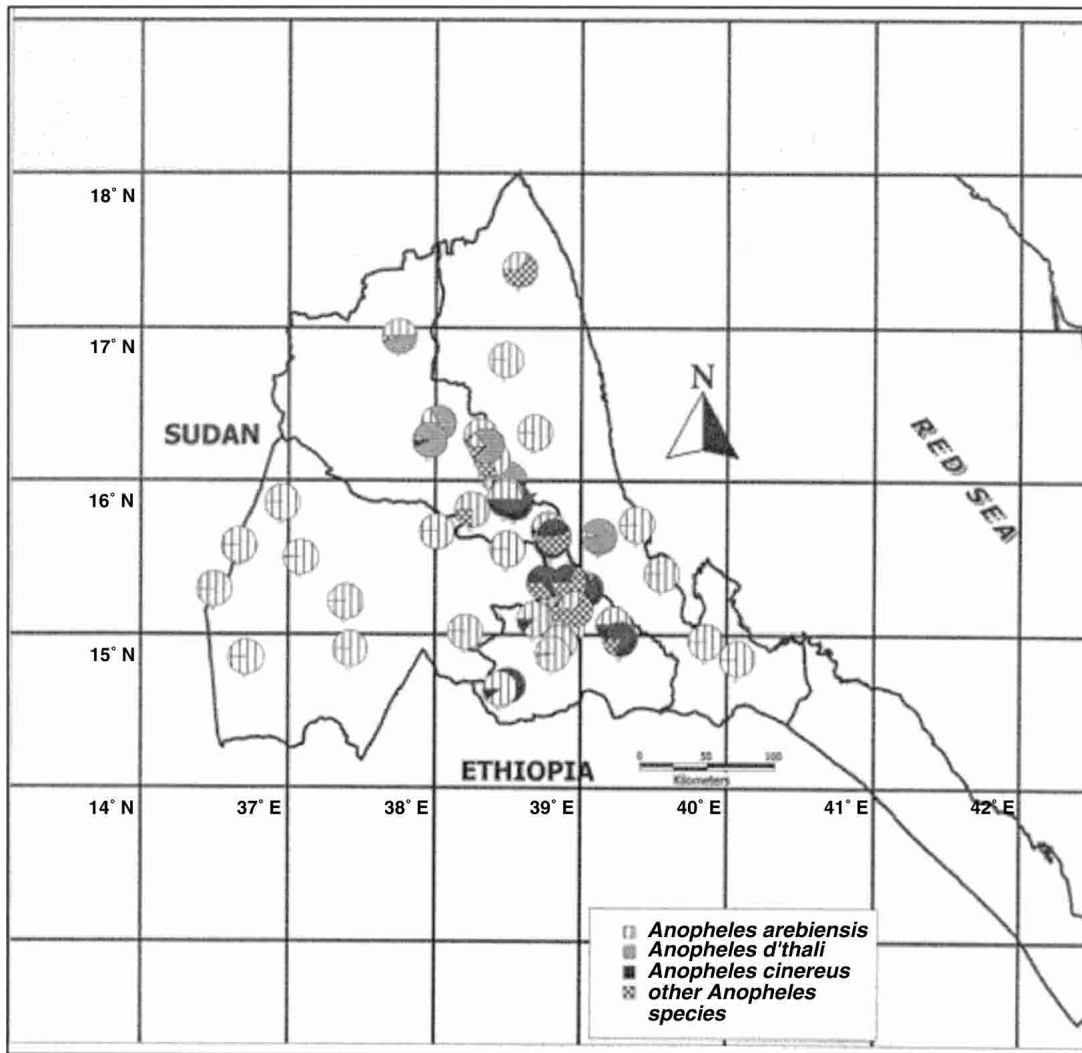


FIGURE 3. Spatial distribution of *Anopheles* species in Eritrea. The greatest diversity in *Anopheles* species was observed in the high altitude areas of the country, which encompasses the Debub and Anseba zones.

DISCUSSION

A total of 13 anopheline species were collected from indoor resting sites representing the ecologic diversity present in Eritrea. The country can be divided roughly into five ecologic

zones, i.e., western lowlands, western escarpments, central highlands, eastern escarpments, and coastal plains. Each of these zones present specific ecologic conditions arising from differences in elevation, rainfall, and temperature, which favor the survival of the wide range of anopheline species. In-

TABLE 3

Summary of the total number of *Anopheles* mosquitoes collected in 59 villages sampled both in the first and second years of the countrywide survey in Eritrea*

Zone	Year of study	<i>An. gambiae s.l.</i>	<i>An. cinereus</i>	<i>An. d'thali</i>	<i>An. squamosus</i>	<i>An. rhodesiensis</i>	<i>An. rupicolus</i>	<i>An. garnhami</i>	<i>An. wellcomi</i>	<i>An. demeilloni</i>	Total
Anseba	Year 1	206	1	160	0	0	8	0	0	3	378
	Year 2	85	3	9	0	0	1	0	0	0	98
Debub	Year 1	11	11	1	0	1	0	3	0	1	28
	Year 2	93	1	0	0	0	0	0	0	0	94
Gash-Barka	Year 1	296	1	0	0	0	0	0	0	0	297
	Year 2	625	0	0	0	0	0	0	0	0	625
NRS	Year 1	49	0	0	0	0	0	0	0	0	49
	Year 2	18	0	0	0	0	0	0	2	0	20
Maekel	Year 1	3	0	0	1	0	0	0	0	0	4
	Year 2	5	0	0	0	0	0	0	0	0	5

* The densities of *An. gambiae s.l.* in the two sampling periods were not significantly different. NRS = Northern Red Sea.

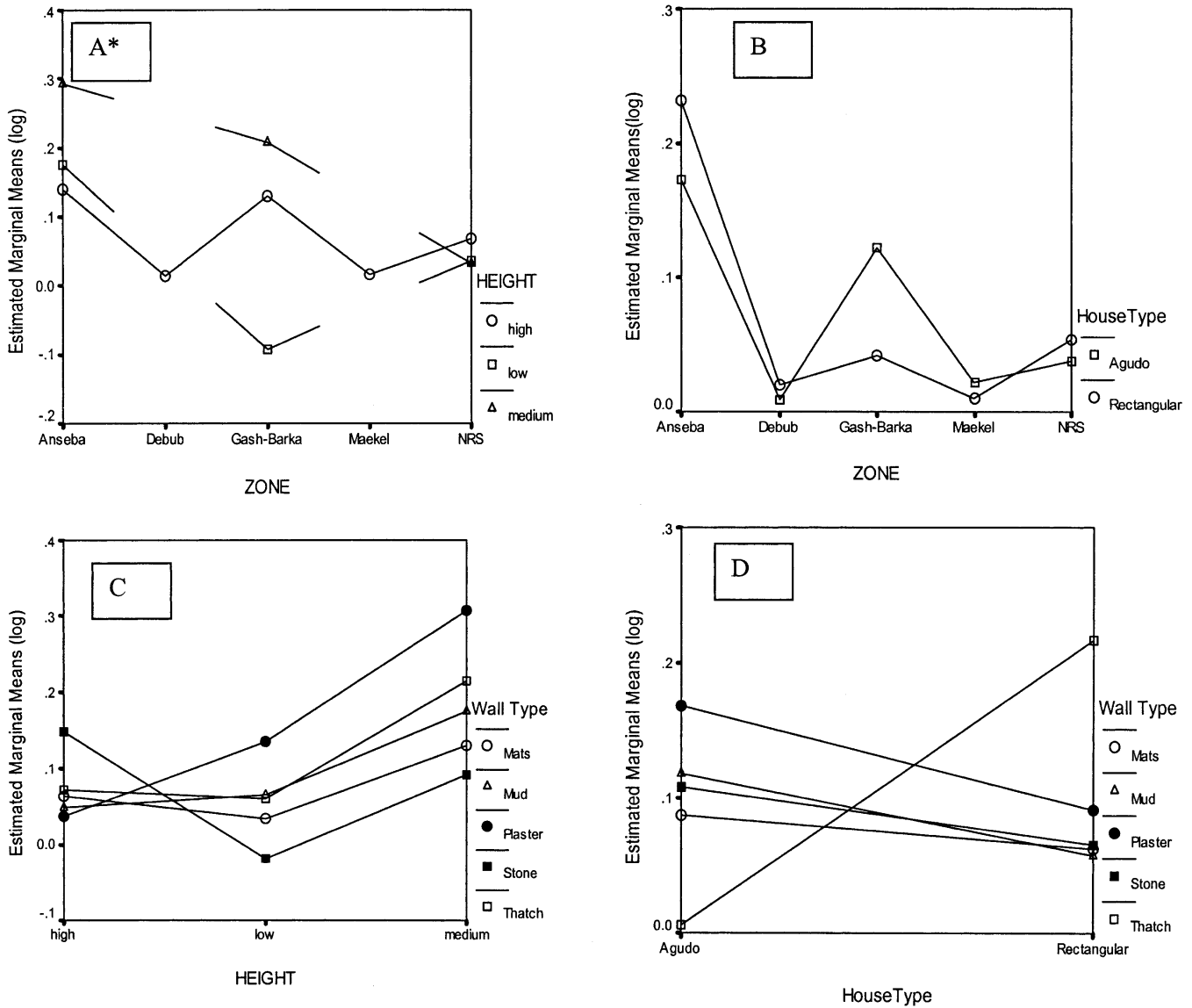


FIGURE 4. Plots of the two-way analysis of variance interactions between **A**, Zone and elevation; **B**, Zone and house type; **C**, Elevation and wall type; and **D**, House type and wall type for *Anopheles arabiensis* in Eritrea. The y-axes show estimated means of *An. arabiensis* adjusted for other factors. *No observations for some combination of the factors. NRS = Northern Red Sea.

trazonal variability in ecologic conditions would further explain the diversity in anopheline species composition observed in specific zones such as the Anseba and Debub zones. *Anopheles d'thali* and *An. cinereus* were the second and third

most abundant species. The low sporozoite infection rate of 0.33% determined in the present study may explain the low levels of malaria endemicity experienced in the country. However, coupled with high biting rates at specific times of the year and vector bionomics, appreciable levels of malaria in the population could be maintained.¹¹ Because *An. d'thali* and *An. cinereus*, which were not previously incriminated as vectors of malaria in Eritrea, were positive for *P. falciparum* circumsporozoite antigens, this suggests that there is need to monitor the abundance of these two species in space and time. The changes in ecologic conditions associated with human influences and global climate changes may affect the abundance and possibly the vector potential of such rare species.

The PCR assays on more than 30% of the *An. gambiae s.l.* specimens indicated that *An. arabiensis* was the only member of the *gambiae* complex present in Eritrea. This result is consistent with findings from preliminary studies conducted in

TABLE 4

Multivariate regression summary for the dependent variable, mean *Anopheles arabiensis* density*

Variables	Regression coefficients	Standard error	t	P
Constant	-0.384	0.152	-2.525	0.012
Rainfall	-0.345	0.016	-3.831	<0.001
Average NDVI	0.023	0.001	0.152	0.879
Maximum NDVI	0.097	0.003	0.500	0.618
Minimum NDVI	0.248	0.003	1.789	0.075

* Number of observations = 302; R² = 0.058; adjusted R² = 0.046; NDVI = normalized difference vegetation index.

five villages in western Eritrea, along the border with Ethiopia and Sudan, in which *An. arabiensis* was documented as the only member of the *An. gambiae* complex through cytogenetic studies.¹² *Anopheles arabiensis* has been documented as the predominant member of the *An. gambiae* complex and the main vector of *P. falciparum* malaria in neighboring provinces of Ethiopia and the Sudan.^{13,14} Eritrea generally is semi-arid and receives scanty rainfall (<500 mm per year), within a short rainy season that lasts no more than three months. Previous findings on the bionomics and habitat preferences show that *An. arabiensis* would normally predominate under such dry climatic conditions.¹⁵⁻¹⁷ In Eritrea, this species is associated with river systems, with breeding maintained in stream bed pools throughout the year and in transient pools such as roadside ditches during the rainy season.¹⁸

Almost 90% of the total mosquitoes were sampled from only 10% of the houses. This heterogeneity in distribution may have important operational significance because vector control operations would be designed to target villages within zones where high densities of malaria vectors are found. For example, a greater risk of malaria infection and intensity of malaria transmission is likely in specific sites in the Gash-Barka zone based on the vector density recorded. A risk-stratified approach to vector control would save on effort and cost in situations where resources are limited as is the case in most malaria-endemic areas in sub-Saharan Africa. The significant interactions between house-related factors revealed by the ANOVA model are useful for designing sampling plans and show the need to focus on house type in implementing vector control measures.

Elevation greatly influenced mosquito densities due to its modifying effect on temperature and humidity. Anopheline mosquitoes were present at altitudes greater than 2,000 meters, indicating that such areas would not remain free of malaria in the light of the modifying effects to the ecosystem associated with human influences on both local and global scale.⁵ Fairly high densities of anophelines also were sampled at altitudes between 1,400 and 1,800 meters above sea level. These fringe areas may be prone to malaria epidemics since the highest density of the non-immune population in Eritrea is also found within these areas.

No significant association was found between NDVI summaries, rainfall, and *An. arabiensis* density in the regression model. This could be attributed to the complex nature of interactions between these ecologic variables, which may have specific time lags that may not have been captured by the present analyses. Rainfall independently was significantly associated with the *An. arabiensis* population. This occurrence confirms the seasonal nature of malaria transmission in Eritrea, which coincides with the one short rainy season experienced in all ecologic regions of the country. In the drier regions of Africa, such as the Sahelian belt, it has been observed that even relatively small differences in rainfall and soil moisture content can influence substantial changes in the ecology of an area and consequent differences in the composition of mosquito vector species.¹⁹ For instance, chromosomal variants of *An. gambiae* have been shown to be commonly associated with prevailing ecologic conditions of a given region.²⁰ The small area variations in rainfall amounts in the semi-arid situation experienced in Eritrea could therefore significantly affect mosquito density and composition. It is likely that systematic monitoring of rainfall amounts under such

semi-arid conditions can provide a sensitive surveillance tool for forecasting malaria transmission in the country.

The present study is the first to provide information on the vector species diversity in Eritrea on a countrywide scale. The intra-zonal variations observed in the present study show that heterogeneities in risk status have to be addressed even within a small ecologic area. Strengthening of vector surveillance mechanisms to monitor surges in vector densities would be central in making informed vector control decisions.

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Authors' addresses: Josephat Shililu, National Malaria Control Program, Division of Communicable Diseases Control, Ministry of Health, PO Box 212 Asmara, Eritrea and International Centre of Insect Physiology and Ecology, PO Box 30772, Nairobi, Kenya. Tewolde Ghebremeskel, Solomon Mengistu, Helen Fekadu, and Meshari Zerom, National Malaria Control Program, Division of Communicable Diseases Control, Ministry of Health, PO Box 212 Asmara, Eritrea. Charles Mbogo and John Githure, International Centre of Insect Physiology and Ecology, PO Box 30772, Nairobi, Kenya, Telephone: 254-2-861-680, Fax: 254-2-860110. Weidong Gu and Robert Novak, Illinois Natural History Survey, 607 E. Peabody, Champaign, IL, Telephone: 217-333-1186, Fax: 217-333-2359. John C. Beier, Department of Epidemiology and Public Health, University of Miami School of Medicine, Highland Professional Building, 1801 NW 9th Avenue, Suite 300 (D-93), Miami, FL 33136, Telephone: 305-243-2977, Fax: 305-243-3324.

Reprint requests: Josephat Shililu, National Malaria Control Program, Division of Communicable Diseases Control, Ministry of Health, PO Box 212, Asmara, Eritrea. Telephone: 291-1-125-529, Fax: 291-1-122-899 or 291-1-125835, E-mail: shililu@gemel.com.er.

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