Variability in natural populations of *Anopheles sacharovi* (Diptera: Culicidae) from southeast Anatolia, revealed by morphometric and allozymic analyses

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ABSTRACT: Four populations of *Anopheles sacharovi* Favre occurring in different ecological subregions at altitudes between 353-1,126 m in the Sanliurfa Province of southeast Turkey were compared using morphometric and allozyme analyses. Four allozyme loci were assessed for genetic differentiation among samples from four localities. The similarity phenogram obtained from the allozyme data showed that populations at Birecik and Sandi branched as a separate group from the Pamuklu and Gedik populations. The Gedik population at the highest altitude (1,126 m) was clustered as a separate branch when linear measurements of 63 morphological characteristics were examined. The UPGMA phenogram also showed that Pamuklu and Sandi formed a cluster while Birecik and Gedik formed separate groups. ***Journal of Vector Ecology*** **30 (2): 206-212. 2005.**

Keyword Index: Anopheles sacharovi, geographical variation, traditional morphometrics, allozyme electrophoresis.

INTRODUCTION

Turkey is situated on the edge of the subtropical zone in which certain vector-borne diseases are prevalent at endemic and occasionally epidemic proportions (Ramsdale and Haas 1978). Historically, malaria has been one of the most important vector-borne diseases in Anatolia (Merdivenci 1984, Akdur 1999). Malaria is not evenly distributed in the country and is endemic mainly in the provinces of the Southeastern Anatolia Irrigation Project (GAP) and a portion of eastern Anatolia (Kasap et al. 2000). At present, 23% of the total population of Turkey still lives in those provinces where malaria is endemic. In 2001, 84% of malaria cases that occurred in Turkey were recorded from the GAP area (Anonymous 2002).

In southeastern Anatolia, malaria is focused in Sanliurfa province. The geographical, ecological, and socio-economical characteristics of the province play important roles in the distribution and epidemics of malaria (Alten et al. 2003). This disease has become a greater threat in recent years because the economic opportunities in the provinces of the GAP have attracted human populations to Sanliurfa. Malaria has been controlled and/or supressed in Sanliurfa between 2001 and 2003, yet based on a study of 1,306 individuals between 1999 and 2002, the rate of prevalance of malaria in Sanliurfa was between 1.90 to 8.29 % of the total population of 1.3 million in Sanliurfa (Alten et al. 2003).

Previous studies have shown that there are thirteen *Anopheles* species recorded in Turkey (Ramsdale et al. 2001). Among them, *Anopheles sacharovi* Favre is the most common malaria vector followed by *Anopheles maculipennis* Meigen, *Anopheles claviger* Meigen, and *Anopheles superpictus* Grassi (Kasap et al. 1987, Kasap 1990, Özer et al. 2001). In the past, three indigenous parasite species have been reported in Turkey: *Plasmodium malaria, P. falciparum,* and *P. vivax*. Currently, although occasionally imported cases of *P. falciparum* are observed, all indigenous cases of malaria are *P. vivax* (Anonymous 2002, Alten et al. 2003).

Turkey has a variety of geographical, climatic, geological, and ecological conditions giving rise to a proliferation of different mosquito species. Furthermore, these differentiations may have also caused inter/intraspecific variations that are revealed by morphometric and allozymic analyses as in other insect species (Belen et al. 2004). The main objective of the current study was to investigate the effects of altitude as an important geographical factor on local populations of *An. sacharovi*. In light of previous studies done in the region (Alten et al. 2003, Belen et al. 2004), we have focused on four different eco-regions that are classified gradually in this very important endemic malaria site of Turkey. It was hypothesized that habitat differences associated with altitude may affect the morphological characteristics in a multivariate way relating to allozyme variability.

MATERIALS AND METHODS

The study area

The study was carried out in Sanliurfa Province (37° 09' N; 38° 47' E), SE Anatolia, Turkey from May 2001 to October 2002. Sanliurfa Province, which is 18,500 km^2, is located in the western part of the southeastern Anatolia region near the border of Syria. The city of Sanliurfa lies at 550 m and has a semi-arid climate with four distinct seasons: a very hot and dry summer (June-August; 31.5°C, 3.20 mm rainfall), a warm, wet autumn (September-October; 20.7°C, 14.14 mm rainfall), a moderately warm and rainy winter (December-February; 7.5°C, 71.33 mm rainfall), and a warm and rainy spring (March-May; 17.0°C, 37.03 mm rainfall).

Based on the results of a previous study (Alten et al. 2003), four localities at different elevations were selected. The Birecik (BRC) region (37°01'N, 37°57'S) is the lowest region with an altitude of 353 m located in the west of the province, and the mean temperature is higher than that of the

north. The Sandi (SND) region (37°19'N, 39°34'S) has an altitude of 682 m. Pamuklu (PMK) region (37°36'N, 39°19'S) is at 743 m and Gedik (GDK) (37°45'N, 39°40'S) is the highest mountainous and rural area with an altitude of 1,126 m. The incidence of malaria ranged from 1.6 % to 9.8 % among these locations (Alten et al. 2003). The major plant species found in the region are: *Astragalus aleppicus* Boiss., *Astragalus aduncus* Willd., *Onobrychis crista-galli* (L.) Lam., *Adonis dentata* Del., *Medicago orbicularis* (L.) Bart. *Eryngium criticum* Lam., *Trifolium pillulare* Boiss., *Linum mucronotum* Bertol., and *Salvia brachyntha* (Brodz.) Pobed. The main agricultural products of the Sanliurfa region are; *Pistacia vera* L., *Vitis sylvestris* Gmelin, *Triticum aestivum* L., *Oryza sativa* L., and *Gossipium spp*. Apart from agriculture, livestock breeding is the major source of income in the area. Sheep are the most common livestock but cattle and chickens can also be found in some areas.

Mosquito sampling

An. sacharovi adults were collected by aspirating from houses and barns and from animal-bait traps made from polyester netting and baited with cows that were placed near houses of current or past malaria patients in each locality. Collections were also made using CDC miniature light - traps (John W. Hock Co. Florida, U.S.A.) during the summers of 2001 and 2002 (August-September). On each trapping night, four to six light traps were placed in each of the sampling localities. Houses and barns used as sampling stations varied from two-story cement block enclosures to simple brick, stone, or cement houses with basements, cellars, caves, or barns for keeping poultry or livestock. Field-collected live females (fed or gravid) were transported to the laboratory in polyethylene containers that were kept on ice for the later morphometric and allozyme studies (Urbanelli et al. 2000). Taxonomic identification was made using the keys and descriptions of Ross and Roberts (1943), Dubose and Curtin (1965), Glick (1992), Darsie and Samanidou (1997) and Schaffner et al. (2001). Identifications were reconfirmed using voucher specimens of *An. sacharovi* from Sanliurfa, Turkey. After identification, *An. sacharovi* females were separated from the other live flies and introduced into rearing cages (50x50x50 cm). About 400 females were collected from study areas, and 92 of them were randomly chosen for morphometric analyses and 250 for allozyme analyses.

Laboratory studies

Electrophoresis. The thoraces of females were ground up and homogenates were kept at -80° C until they were used for electrophoresis. Four enzyme systems, MDH (malate dehydrogenase, EC 1.1.1.37), PGI (phosphoglucoisomerase, EC 5.3.1.9), EST (esterase, EC 3.1.1), and HK (hexokinase, EC 2.7.1.1), were studied by horizontal starch-gel electrophoresis. Two enzyme systems (EST and PGI) were studied using the Tris-citrate, pH 7.0 buffer system (Shaw and Prasad 1970, Hillis and Moritz 1990). MDH was studied using the Tris-HCl, pH 8.6 buffer system (Shaw and Prasad 1970), while HK was studied using the Tris-malate-EDTA, pH 7.4 buffer system (Shaw and Prasad 1970). Sample and gel preparation and experimental conditions were similar to those of Kandemir and Kence (1995). Statistical analysis of electrophoretic data was performed using the computer program BIOSYS-1 (Swofford and Selander 1981).

Morphometric Analysis. All the specimens were screened for the presence of known parasites in order to prevent possible traumatic variations affecting the morphometric data (Mayr and Ashlock 1991). The body parts were then removed from each specimen with forceps and mounted on slides. All slides were photographed using a Leica MZ-7.5 stereoscopic zoom dissection microscope with a DC-300 digital camera system, digitized and archived. A total of 63 characters were measured using TPSdig (Rohlf 2003) software as follows: **1)** length of wing (a-o), **2)** width of wing, **3)** length of subcosta (b-d), **4)** distance between f-e points on r_2 vein, **5)** length of R-M (g-h), **6)** distance between n-k points on the Cu_2, **7)** distance between k-j points on the Cu_2 vein, **8)** distance between k-l points, **9)** distance between h-m points on the m_3 vein, **10)** length of M-Cu_1 (i-j) (Figure 1), **11)** length of haltere, **12)** length of the apical part of haltere, **13-36)** [**I)** length of femur, **II)** Width of femur, **III)** length of tibia, **IV)** length of 1st segment of tarsus, **V)** length of 2nd segment of tarsus, **VI)** length of 3rd segment of tarsus, **VII)** length of 4th segment of tarsus, **VIII)** length of 5th segment of tarsus (fore, mid and hind legs 3 x 8 = 24 characters)], **37)** length of head, **38)** width of head, **39)** length of compound eyes, **40)** length of clypeus, **41)** length of palpus **42)** length of 1st and (plus) 2nd segment of palpus, **43)** length of 3rd segment of palpus, **44)** length of 4th segment of palpus, **45)** length of 5th segment of palpus, **46)** length of proboscis, **47)** length of label, **48-62)** length of total antenna, [**I)** length of antennal segments (total 14 characters)], **63)** length of cercus. For paired organs, those on the right side were measured (Aytekin and Cagatay 2002). The collected data were tested for allometry by Huxley's model and transformed into natural logarithmic form.

For statistical analysis of morphology in terms of size morphometry, the data were transformed into natural logarithmic form (Debat et al. 2003). The data were discriminated using a discriminant multigroup function analysis (Canonical variate analysis CANOVAR) by Syn-tax 2000 (Podani 2001) package (Exeter-Software, U.S.A.). The arithmetic means of the 63 morphometric measurements were clustered by Ward's method with PAST 2004 software package (Hammer et al. 2004). The significant differences among characters were also tested by one way ANOVA (Sokal and Rohlf 1981).

RESULTS

Electrophoresis results

Allelic frequencies and genetic differentiation of all populations for the four loci are given together with their standard errors in Table 1. The similarity phenogram obtained from these data are presented in Figure 2. From Table 1 it appears that although the differentiation is not exact, the BRC and SND constitute one group while PMK and GDK another.

Morphometric results

Individual distribution and clusters obtained from the

Table 1. Values of the genetic differentiation based on allozymes for four different population of *Anopheles sacharovi* collected from four different altitudes (Birecik (BRC) 353 m, Sandi (BRC) 682 m, Pamuklu (PMK) 743 m, and Gedik (GDK) 1,126 m), Sanliurfa, southeastern Turkey (May 2001-October 2002).

	Allele frequencies													Mean heterozygosity				
	Hk					*Mdh-1*	*Est-3*				*Pgi-1*			Mean sample size	Mean number of alleles	Percentage of loci		
Population	A	B	C	D	E	A	A	B	C	D	A	B	C	per locus	per locus	polymorphic*	Observed	Expected (Hardy-Weinberg**)
BRC	0.188	0.250	0.188	0.208	0.167	1.000	0.500	0.300	0.114	0.086	0.500	0.338	0.162	19.5 (6.5)	2.5 (.7)	50.0	303 (.189)	347 (.157)
SND	0.241	0.172	0.345	0.172	0.069	1.000	0.443	0.414	0.114	0.029	0.500	0.233	0.267	25.7 (8.1)	2.5 (.7)	50.0	307 (.184)	338 (.153)
PMK	0.065	0.391	0.152	0.304	0.087	1.000	0.538	0.308	0.128	0.026	0.500	0.486	0.014	22.5 (7.2)	2.5 (.7)	50.0	298 (.184)	310 (.142)
GDK	0.100	0.250	0.200	0.325	0.125	1.000	0.409	0.318	0.227	0.045	0.500	0.297	0.203	16.0 (5.6)	2.5 (.7)	50.0	323 (.192)	351 (.158)

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
** Unbiased estimates (see Nei 1978).

morphometric analysis are shown in Figure 3. For morphometrics, although the personal measurement error ($æ_p$) (Arnqvist and Mårtensson 1998) as well as the standardization and transformation procedure errors are kept at a minimum, the unexpected intravariation is still seen in four characters (length of hind leg segment, wing length, length of cerci, and length of haltere). The errors in accuracy and precision of the data are mostly due to ambiguity of the reference points of these characters, resulting in shifts during measurement and/ or the difficult procedure in the preparation and processing of these body parts. Errors due to two-dimensional viewing of a three-dimensional object can possibly be another reason. Analysis conducted taking the measurement errors into consideration clearly show that GDK (1,126 m) forms a distinct group from the other three regions (BRC, 353 m; SAND, 682 m; PMK, 743 m) for the 63 characters measured along the first two canonical axes (Figure 3). The differences between the local populations of *An. sacharovi* are clear when all populations are considered. SND and PMK populations are the closest ones along the first two axes. When an UPGMA tree is constructed, Gedik and Birecik populations show distinct group patterns independently from altitude and related factors (Figure 4). Means with standart deviation are also calculated for each character and some of them show significant differences (Table 2).

DISCUSSION

The distribution of *An. sacharovi* is from Italy to the former U.S.S.R. and China, and from Jordan to Israel, Syria, Iraq, Iran, Cyprus, and Turkey (Merdivenci 1984). Its range in Turkey includes the western, southern, and northern coastal plains and extends to the central plateau (Postiglione et al. 1973, Merdivenci 1984, Ramsdale et al. 2001). Increasing altitude and longitude may influence climatic factors such as precipitation and temperature that are important determinants relative to the abundance, life-history, and morphology of mosquitoes (Gleiser et al. 2000).

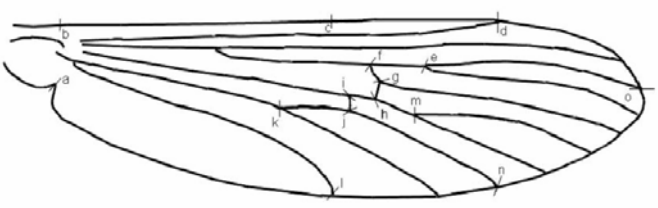

Figure 1. Characters used in morphometric measurements of wing.

The results presented here indicate that there are significant differences among populations. When allozyme data and phenograms are examined it was seen that BRC and SND branched as a separate group from the PMK and GDK populations (Figure 2, Table 1). It is difficult to identify the possible mechanisms of the genetic variability among these populations with these data. Urbanelli et al. (2000) showed that although there was genetic homogenity on a large scale for *Aedes albopictus*, genetic differentiation between closely situated sites (10-20 km) resulted from genetic drift, in line with low dispersal and founder effects, thus showing that isolation by distance could be effective even at close distances for mosquitoes. However, since no clear pattern of isolation by distance was observed (for example SND and PMK branched separately althought they were almost at the same altitude) human effects such as effective insecticide application appears to be more important.

When analyzed by one-way ANOVA, the populations were found to be significantly different for 45 morphological characters (Table 2). The differences among the four populations primarily occurred in the wing, leg, head and haltere characters. This may be associated with factors such as climatic, ecological, or socio-biological effects. When whole size differences were analyzed by UPGMA, GDK and BRC grouped separately and PMK and SND clustered together (Figure 4). Although BRC and GDK populations live at different altitudes and climates, they have a similar morphology. Therefore, size similarity and dissimilarity does not seem to have an obvious environmental explanation like climatic differences brought on by altitude. This situation again hints towards some ideas like differential insecticide applications which might result in bottlenecks as a possible explanation for size variation. Size differences showed somewhat different results when analyzed by CANOVAR. This may indicate a directional selection or genetic drift, especially on the GDK population. Only the GDK population was found to be significantly different from the other populations (Figure 3). Because GDK is situated at the highest altitude, climatic factors could explain this difference. Wing

Table 2. Arithmetic mean (± standard deviation) for the 45 morphological characters that show significant differences $p < 0.05$ among four populations of *Anopheles sacharovi* collected from four altitudes (Birecik (BRC) 353 m, Sandi (SND) 682 m, Pamuklu (PMK) 743 m, and Gedik (GDK) 1,126 m), Sanliurfa, southeastern Turkey (May 2001- October 2002).

Characters (10^{-1} mm)	BRC	SND	PMK	GDK	*p*
1. Length of wing	39.78 ± 3.38	38.07 ± 2.86	37.55 ± 2.29	41.85 ± 2.10	0.000
2. Width of wing	10.27 ± 0.74	9.83 ± 0.77	9.69 ± 0.53	10.27 ± 0.69	0.000
3. Length of subcosta	22.97 ± 2.15	21.71 ± 1.96	21.31 ± 1.86	23.39 ± 1.43	0.000
4. Distance between f-e points on r_2 vein	6.24 ± 0.56	6.12 ± 0.63	6.10 ± 0.49	6.61 ± 0.33	0.003
5. Distance between n-k points on Cu_2 vein	16.37 ± 1.25	16.00 ± 1.10	15.8 ± 0.83	17.15 ± 0.86	0.000
6. Distance between k-j points on Cu_2 vein	5.82 ± 0.54	5.47 ± 0.41	5.53 ± 0.35	6.19 ± 0.47	0.000
7. Distance between k-l points	6.28 ± 0.51	6.00 ± 0.41	6.00 ± 0.45	6.62 ± 0.43	0.000
8. Distance between h-m points on m_3 vein	6.10 ± 0.57	6.40 ± 0.54	6.60 ± 0.44	7.02 ± 0.36	0.002
9. Length of haltere	4.16 ± 0.31	3.94 ± 0.30	3.98 ± 0.27	4.24 ± 0.22	0.001
10. Length of the apical part of halter	1.95 ± 0.24	1.83 ± 0.19	1.77 ± 0.14	2.02 ± 0.18	0.000
11. Length of fore femur	21.01 ± 1.29	19.94 ± 1.40	19.87 ± 1.23	21.37 ± 1.42	0.000
12. Width of fore femur	0.99 ± 0.15	0.83 ± 0.17	0.86 ± 0.15	0.89 ± 0.12	0.003
13. Length of fore tibia	24.43 ± 1.54	23.50 ± 1.85	23.17 ± 1.16	25.28 ± 1.72	0.000
14. Length of 1st segment of fore tarsus	18.61 ± 1.16	17.93 ± 1.37	17.79 ± 0.99	18.92 ± 1.10	0.004
15. Length of 2nd segment of fore tarsus	6.85 ± 0.51	6.67 ± 0.55	6.67 ± 0.40	7.12 ± 0.56	0.014
16. Length of 3rd segment of fore tarsus	4.71 ± 0.41	4.59 ± 0.54	4.42 ± 0.27	4.90 ± 0.38	0.002
17. Length of 4th segment of fore tarsus	2.82 ± 0.24	2.65 ± 0.26	2.66 ± 0.16	2.87 ± 0.23	0.002
18. Length of 5th segment of fore tarsus	2.00 ± 0.12	1.90 ± 0.17	1.87 ± 0.14	1.97 ± 0.13	0.005
19. Length of mid femur	25.20 ± 1.63	24.08 ± 0.83	23.61 ± 1.57	25.44 ± 1.69	0.001
20. Width of mid femur	0.76 ± 0.11	0.66 ± 0.09	0.70 ± 0.10	0.74 ± 0.09	0.004
21. Length of mid tibia	25.89 ± 1.82	25.25 ± 2.05	24.36 ± 1.53	26.61 ± 1.53	0.000
22. Length of 1st segment of mid tarsus	5.77 ± 0.38	5.58 ± 0.42	5.51 ± 0.34	6.04 ± 0.57	0.000
23. Length of 4th segment of mid tarsus	3.60 ± 0.28	3.43 ± 0.26	3.40 ± 0.26	3.58 ± 0.23	0.014
24. Length of 5th segment of mid tarsus	2.20 ± 0.14	2.05 ± 0.16	2.04 ± 0.15	2.10 ± 0.12	0.001
25. Length of hind femur	25.74 ± 1.62	24.54 ± 2.00	24.19 ± 1.36	26.29 ± 1.80	0.000
26. Width of hind femur	0.66 ± 0.09	0.60 ± 0.10	0.62 ± 0.08	0.67 ± 0.06	0.033
27. Length of hind tibia	27.30 ± 2.06	26.39 ± 2.24	25.71 ± 1.44	28.18 ± 1.54	0.000
28. Length of 1st segment of hind tarsus	31.96 ± 2.34	30.63 ± 2.74	30.60 ± 2.11	32.74 ± 2.12	0.005
29. Length of 2nd segment of hind tarsus	13.03 ± 0.94	12.33 ± 1.02	1.47 ± 0.81	13.32 ± 0.93	0.001
30. Length of 3rd segment of hind tarsus	9.61 ± 0.83	9.17 ± 1.17	9.13 ± 0.64	9.95 ± 0.69	0.005
31. Length of 4th segment of hind tarsus	5.79 ± 0.38	5.27 ± 0.65	5.30 ± 0.38	5.56 ± 0.58	0.002
32. Length of 5th segment of hind tarsus	2.87 ± 0.18	2.64 ± 0.23	2.64 ± 0.22	2.78 ± 0.39	0.010
33. Length of head	7.72 ± 0.42	7.48 ± 0.45	7.39 ± 0.28	7.59 ± 0.45	0.044
34. Width of head	7.35 ± 0.34	7.03 ± 0.36	6.92 ± 0.35	7.04 ± 0.38	0.001
35. Length of compound eyes	4.69 ± 0.19	4.56 ± 0.20	4.52 ± 0.15	4.62 ± 0.29	0.011
36. Length of clypeus	3.12 ± 0.29	2.83 ± 0.26	2.88 ± 0.20	2.86 ± 0.28	0.001
37. Length of palpus	21.15 ± 1.67	20.47 ± 1.55	20.25 ± 1.55	21.74 ± 1.39	0.007
38. Length of 3rd segment of palpus	7.94 ± 0.65	7.57 ± 0.62	7.44 ± 0.65	7.98 ± 0.68	0.013
39. Length of 5th segment of palpus	2.16 ± 0.22	2.11 ± 0.16	2.12 ± 0.20	2.30 ± 0.19	0.003
40. Length of proboscis	22.50 ± 1.16	21.63 ± 1.32	21.22 ± 1.20	22.65 ± 1.07	0.000
41. Length of labellum	2.02 ± 0.11	1.93 ± 0.14	1.87 ± 0.11	1.96 ± 0.13	0.001
42. Length of 1st antennal segment	0.93 ± 0.42	0.76 ± 0.08	0.81 ± 0.12	0.77 ± 0.11	0.040
43. Length of 12th antennal segment	1.20 ± 0.11	1.13 ± 0.09	1.12 ± 0.08	1.17 ± 0.06	0.011
44. Length of 14 and 15th antennal segment	1.24 ± 0.11	1.18 ± 0.10	1.16 ± 0.10	1.20 ± 0.10	0.032
45. Length of cercus	1.97 ± 0.17	1.76 ± 0.15	1.70 ± 0.18	1.73 ± 0.11	0.000

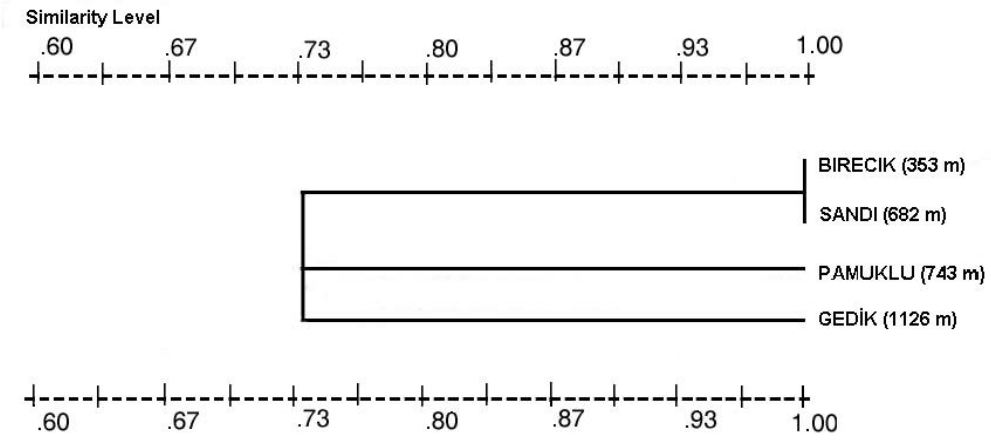

Figure 2. Similarity phenogram obtained from the data of allozymes for four different populations of *Anopheles sacharovi* collected from four altitudes (Birecik (BRC) 353 m, Sandi (SND) 682 m, Pamuklu (PMK) 743 m, and Gedik (GDK) 1,126 m), Sanliurfa, southeastern Turkey (May 2001-October 2002).

length is a determinant of body size because it is a relatively fixed character and is easily measured. Mean wing length value is the highest in GDK populations. An explanation for larger body size might be the influence of developmental temperature on growth. When mosquito larvae develop at lower temperatures, they grow more slowly and complete development at a larger size (Tun-Lin et al. 2000, Alto and Juliano 2001). Gleiser et al. (2000) found that in the floodwater mosquito, *Aedes albifasciatus*, the distribution of wing length varied seasonally and was correlated with both rainfall and breeding site volume. GDK has the highest rainfall and this might explain the observed pattern. We did not find any significant correlation between size variation and allozymes with the incidence of malaria in the region. However, it was interesting to observe the higher vector density in BRC for the future studies.

In conclusion, these morphometric and genetic approaches indicate that geographic variations exist among local populations of *A. sacharovi* in Sanliurfa. The morphometrical studies conducted here are based only on size differences. Future studies could consider size and shape differences using geometric modeling. With increased pressure to use biological control methods, especially those which rely upon altering the genetic structure of a population, greater attention must be given to understanding the life history characteristics of mosquito populations in the field.

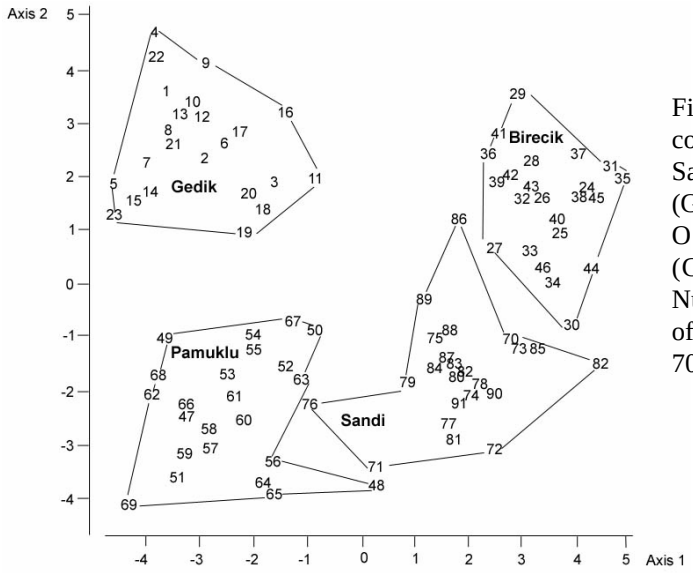

Figure 3. Population distribution of *Anopheles sacharovi* collected at four different altitudes (Birecik (BRC) 353 m, Sandi (SND) 682 m, Pamuklu (PMK) 743 m, and Gedik (GDK) 1,126 m), Sanliurfa, southeastern Turkey (May 2001-October 2002), along the first two principle axes (CANOVAR) based on 63 morphological characters. Numbers within the scatters indicate identification numbers of each individual. 1-23 GDK, 24-46 BRC, 47-69 PMK, and 70-92 SND.

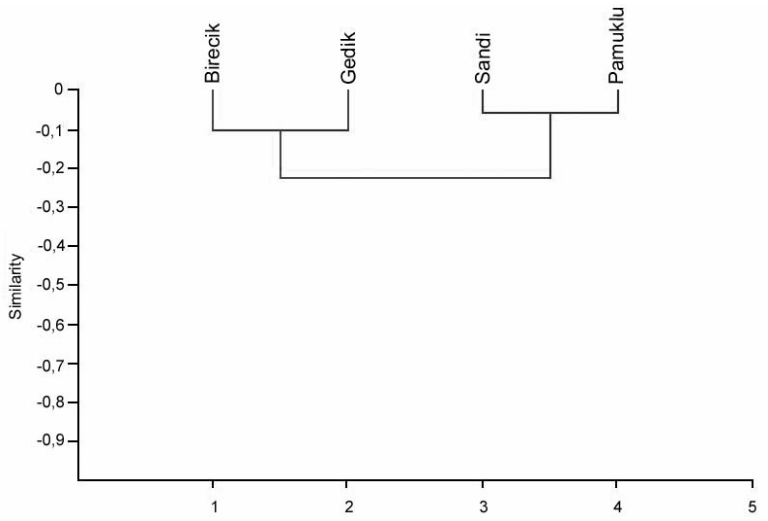

Figure 4. The cluster based on the arithmetic means of 63 morphological characters measured from *Anopheles sacharovi* collected at four different altitudes (Birecik (BRC) 353 m, Sandi (SND) 682 m, Pamuklu (PMK) 743 m, and Gedik (GDK) 1,126 m), Sanliurfa, southeastern Turkey (May 2001-October 2002), (Ward's method).

REFERENCES CITED

Akdur, R. 1999. Malaria. In: Ali Ozcel (ed.) *The epidemiology of malaria*. Turkiye Parasitoloji Dernegi (Turkish). 295 pp.

Alten, B., S. Çağlar, S. Kaynas, and F. Simsek. 2003. Effect of insecticide-treated bednets for malaria control in Southeast Anatolia, Turkey. J. Vector Ecol. 28: 97-107.

Alto, B.W. and S.A. Juliano. 2001. Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): Implications for range expansion. J. Med. Entomol. 38: 646-656.

Anonymous. 2002. Report on the informal consultation on the elimination of residual malaria foci and prevention of re-introduction of malaria, Rabat, Morocco. WHO-EM/MAL/285/E/L, Cairo, Egypt. 24 pp.

Arnqvist, G. and T. Mårtensson. 1998. Measurement error in geometric morphometrics: emprical strategies to assess and reduce its impact on measures of shape. Acta Zool. 44**:** 73-96.

Aytekin A.M. and N. Cagatay. 2002. A phenetic approach to the subgenera of bumblebees (Apidae: Hymenoptera). Mellifera. 2: 60-64.

Belen, A., B. Alten, and A.M. Aytekin. 2004. Altitudinal variation in morphometric and molecular characteristics of *Phlebotomus (Phlebotomus) papatasi* populations. Med. Vet. Entomol. 18: 343-350.

Darsie, R.F. and A. Samanidou-Voyadjoglou. 1997. Keys for the identification of the mosquitoes of Greece. J. Am. Mosq. Contr. Assoc. 13: 231-241.

Debat, V., M. Bégin, H. Legout, and J.R. David. 2003. Allometric and nonallometric components of *Drosophila* wing shape respond differently to developmental temperature. Evolution 57: 2773-2784.

Dubose, P. and T. Curtin. 1965. Identification keys to the adult and larval mosquitoes of the Mediterranean area. J. Med. Entomol. 1: 349-355.

Gleiser, R.M., J. Urrutia, and D.E. Gorla. 2000. Body size variation of the floodwater mosquito *Aedes albifasciatus* in Central Argentina. Med. Vet. Entomol. 14: 38-43.

Glick, J.I. 1992. Illustrated key to the female *Anopheles* of Southwestern Asia and Egypt (Diptera: Culicidae). Mosq. Syst. 24: 125-153.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2004. Paleontological Statistics, ver 1.25.

Hillis, D. M. and C. Moritz. 1990. *Molecular systematics.* Sinauer, Sunderland, MA. U.S.A. 588 pp.

Kandemir, I. and A. Kence. 1995. Allozyme variation in a central Anatolian honeybee (*Apis mellifera* L.) population. Apidologie. 26: 503-510.

Kasap, H., M. Kasap, O. Demirhan, and D. Alptekin. 1987. Development of *Plasmodium vivax* in *Anopheles superpictus* under experimental conditions. Am. J. Trop. Med. Hyg. 27: 241-245.

Kasap, H. 1990. Comparison of experimental infectivity and development of *Plasmodium vivax* in *Anopheles sacharovi* and *Anopheles superpictus* in Turkey. Am. J. Trop. Med. Hyg. 42: 111-117.

Kasap, H., M. Kasap, D. Alptekin, U. Luleyap, and P.R.J. Herath. 2000. Insecticide resistance in *Anopheles sacharovi* Favre in southern Turkey. Bull. Wld. Hlth. Org. 78: 687-692.

Mayr, E. and P.D. Ashlock. 1991. *Principles of Systematic Zoology*, 2nd ed. McGraw-Hill Inc., London.

Merdivenci, A. 1984. *Mosquitoes of Turkey.* Istanbul Universitesi, Cerrahpasa Tip Fakultesi, Publ. No: 3215, Istanbul (Turkish) 354 pp.

Nei, M. 1978. Genetic distance between populations. Am. Nat. 106: 283-292.

Özer, N., B. Alten, and S.S. Çağlar. 2001. Distribution of malaria vectors in Turkey. Proceedings of The Firsth Balkan Conference, Malaria and Mosquito Control.

Serres-Greece. 56-60.
Podani, J. 2001. SYN-TAX 2000 computer programs for data analysis ecology and systematics. + software users manual, Budapest.
Postiglione, M., S. Tabanli, and C.D. Ramsdale. 1973. The *Anopheles* of Turkey. Riv. Parasitol. 34: 127-159.
Ramsdale, C.D. and E. Haas. 1978. Some aspects of epidemiology of resurgent malaria in Turkey. Trans. R. Soc. Trop. Med. Hyg. 72: 570-580.
Ramsdale, C.D., B. Alten, S.S. Çağlar and N. Ozer. 2001. A revised annoted checklist of mosquitoes (Diptera: Culicidae) of Turkey. J. Eur. Mosq. Bull. 9: 18-28.
Rohlf, F.J. 2003. TPSdig. Software. Version 1.38. Ecology and Evolution, SUNY Stony Brook. http://life.Bio.SUNYSB.edu/morph/morph.html.
Ross, E.S. and H.R. Roberts. 1943. Mosquito atlas part II. Eighteen old world Anophelines important to malaria. Am. Entomol. Soc. Acad. Nat. Sci. 39 pp.
Schaffner, F., G. Angel, B. Geoffroy, J.-P. Hervy, A. Rhaiem, and J. Brunhes. 2001. Les moustiques d'Europe, The mosquitos of Europe. CD-ROM. Institut de Recherche pour Development /EID Mediterranée.
Shaw, C.R. and R. Prasad. 1970. Starch gel electrophoresis-a compilation of recipes. Biochem. Genet. 4: 297-320.
Sokal, R.R and F.J. Rohlf. 1981. *Biometry. The principles and practice of statistics in biological research.* W.H. Freeman. 859 pp.
Swofford, D.L. and R.B. Selander. 1981. Biosys-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72: 281-283.
Tun-Lin, W., T.R. Burkot, and B.H. Kay. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. Med. Vet. Entomol. 14: 31-37.
Urbanelli, S., R. Bellini, M. Carrieri, P. Sallicandro and G. Celli. 2000. Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity. 84: 331-337.