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GENETIC DIFFERENCES AMONG *ANOPHELES VESTITIPENNIS* SUBPOPULATIONS COLLECTED USING DIFFERENT METHODS IN CHIAPAS STATE, SOUTHERN MÉXICO

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ABSTRACT. Biting activity and population genetic studies of the malaria vector *Anopheles vestitipennis* were conducted in southern México. Three subpopulations were collected from 2 villages; 2 subpopulations were from the same village, one on human bait and one with an animal-baited trap; the third was collected from a cattle corral in the 2nd village (280 km away SSE). The anthropophilic subpopulation had steady activity with 61% of bites occurring before midnight, significantly different from those of the 2 zoophilic subpopulations, which had 78–82% of bites before midnight and 2 biting peaks, one at 1900–2100 h and the other at 0400–0500 h. Isozyme analysis (13 enzymes) of these subpopulations indicated that differences between the 2 sympatric subpopulations (D = 0.07), collected using 2 different methods, were greater than that between the 2 allopatric ones (D = 0.03). These studies suggest the existence of 2 genetically different subpopulations of *An. vestitipennis* with specific host preferences.

INTRODUCTION

Anopheles vestitipennis Dyar and Knab is a Neotropical anopheline, distributed throughout Central America, as far north as San Luis Potosí, México, south into northern South America (Colombia and Venezuela), and into the Caribbean Islands of Cuba, Jamaica, Hispaniola, Puerto Rico, and the Lesser Antilles (Komp 1942, Lane 1949, Vargas 1958, Wilkerson and Strickman 1990). This species is not considered a major vector of malaria. However, its recent incrimination as a vector of *Plasmodium vivax* and *P. falciparum* in the Lacandón Forest (Loyola et al. 1991, Arredondo-Jiménez 1995[3]) and its extension into Guatemala (Padilla et al. 1992), has raised questions about its role in malaria transmission in other regions, particularly in the Pacific Coastal Plain, where it is abundant (Arredondo-Jiménez 1995[3]).

In the state of Chiapas, México, *An. vestitipennis* is widely distributed, occurring in at least 19 counties (Vargas 1958; Centro de Investigación de Paludismo [CIP], unpublished data). Recent studies conducted in 6 of these counties revealed that vector–human contact is more intense in the northern parts of the state (Lacandón Forest) than in the southern coastal plain. Bloodfeeding studies indicated that preference for humans was not only due to the relative abundance of human hosts, but perhaps to genetically determined host preference (Arredondo-Jiménez 1995[3]).

To test whether mosquitoes collected by different methods (landing on humans, horse-baited trap, and cattle corral collections) are genetically different, we conducted a population genetic study of 3 subpopulations (2 sympatric from the Lacandón Forest and one allopatric from the Tapachula Coastal Plain), using standard isozyme electrophoretic techniques. We also conducted 12-h landing collections to assess differences in biting activity.

MATERIALS AND METHODS

Mosquito collections: Collections were made from February 1992 to December 1993, in 2 villages, Benemérito de las Américas (BENE; 16°31′08″N, 90°39′02″W) located in the Lacandón Forest, and Cosalapa (COSA; 14°37′30″N, 92°16′54″W), located in the Pacific Coastal Plain, within the state of Chiapas, México (Fig. 1). The 2 villages are approximately 280 km apart.

Three subpopulations (A, B, and C) of *An. vestitipennis,* defined by capture method and location, were collected over 12-h periods (1800–0600 h). Subpopulation A was composed of individuals collected while landing on human volunteers in 2 houses from BENE. Catches were made outside houses by 2 pairs of collectors 4 nights per month, each working 6-h shifts. Mosquitoes were collected using hand aspirators during 45-min periods each hour, followed by a 15-min rest period. Results were expressed as mean number of mosquitoes landing per man per hour.

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[3] Arredondo-Jiménez, J. I. 1995. Comparative ecology of allopatric populations of *Anopheles* (*Anopheles*) *vestitipennis* (Diptera: Culicidae). Ph.D. Dissertation. University of California, Davis, CA.

Fig. 1. Geographic distribution of *Anopheles vestitipennis* (shaded area), including the location of the study sites.

In the same village, subpopulation B comprised mosquitoes collected in a horse-baited net trap, which was situated about 35 m and 130 m away from the 2 houses where landing collections were made. Mosquitoes were collected using an oversized nylon net trap baited with a tethered horse inside. The trap was hung 20–30 cm from the ground to allow the entrance of mosquitoes (Bown and Bang 1980). Because we observed that *An. vestitipennis* tended to rest on the net next to the host before and after taking a blood meal, the above technique was modified by hanging the net on a small tree next to the tethered horse. Collections of mosquitoes were made by 5-min searches every 15 min, the results of 3 searches were pooled and expressed as mean number of mosquitoes per hour.

Subpopulation C was collected from COSA next to a cattle corral, with mosquitoes resting intercurrently (Mattingly 1965) on fences or surrounding vegetation before or after a blood meal. Forty-five-minute collections around the corral were expressed as the mean number of mosquitoes per hour.

Differences in biting activity among subpopulations were assessed using 2-factor analysis of variance followed by unequal sample size HSD Tukey tests (Spjotvoll and Stoline 1973). Log(1 + x) transformations of the data were used to normalize values and stabilize the variance (Zar 1984). Adult female mosquitoes were identified as *An. vestitipennis* according to Wilkerson and Strickman (1990) and Orozco (1994[4]).

Isozyme analysis: For enzyme electrophoretic studies, mosquitoes were transported alive to the Centro de Investigación de Paludismo in Tapachula, Chiapas, where they were immediately frozen in liquid nitrogen. They were then sent by air freight to the Department of Entomology at the University of California, Davis, where they were stored at −80°C until processed.

Electrophoretic analysis of soluble enzymes was carried out following techniques described in Eldridge et al. (1986) and Munstermann (1988). Thirteen enzyme loci were analyzed using standard procedures (Steiner and Joslyn 1979): IDH-2 (Enzyme Commission number [E.C.] 1.1.1.42), PGM (E.C. 2.7.5.1), PGI (E.C. 5.3.1.9), MDH-1 (E.C. 1.1.1.37), ME (E.C. 1.1.1.40), HAD (E.C. 1.1.1.30), PGD (E.C. 1.1.1.44), αGPD (E.C. 1.1.1.8), DIA (E.C.

[4] Orozco, A. 1994. Morfología de las etapas en larva de IV estadio, pupa y adulto de *Anopheles* (*Anopheles*) *vestitipennis* Dyar & Knab, 1906 (Diptera: Culicidae) en el sur de Chiapas, México. B.S. Thesis. Universidad Autónoma de Chiapas, Tapachula, Chiapas, México.

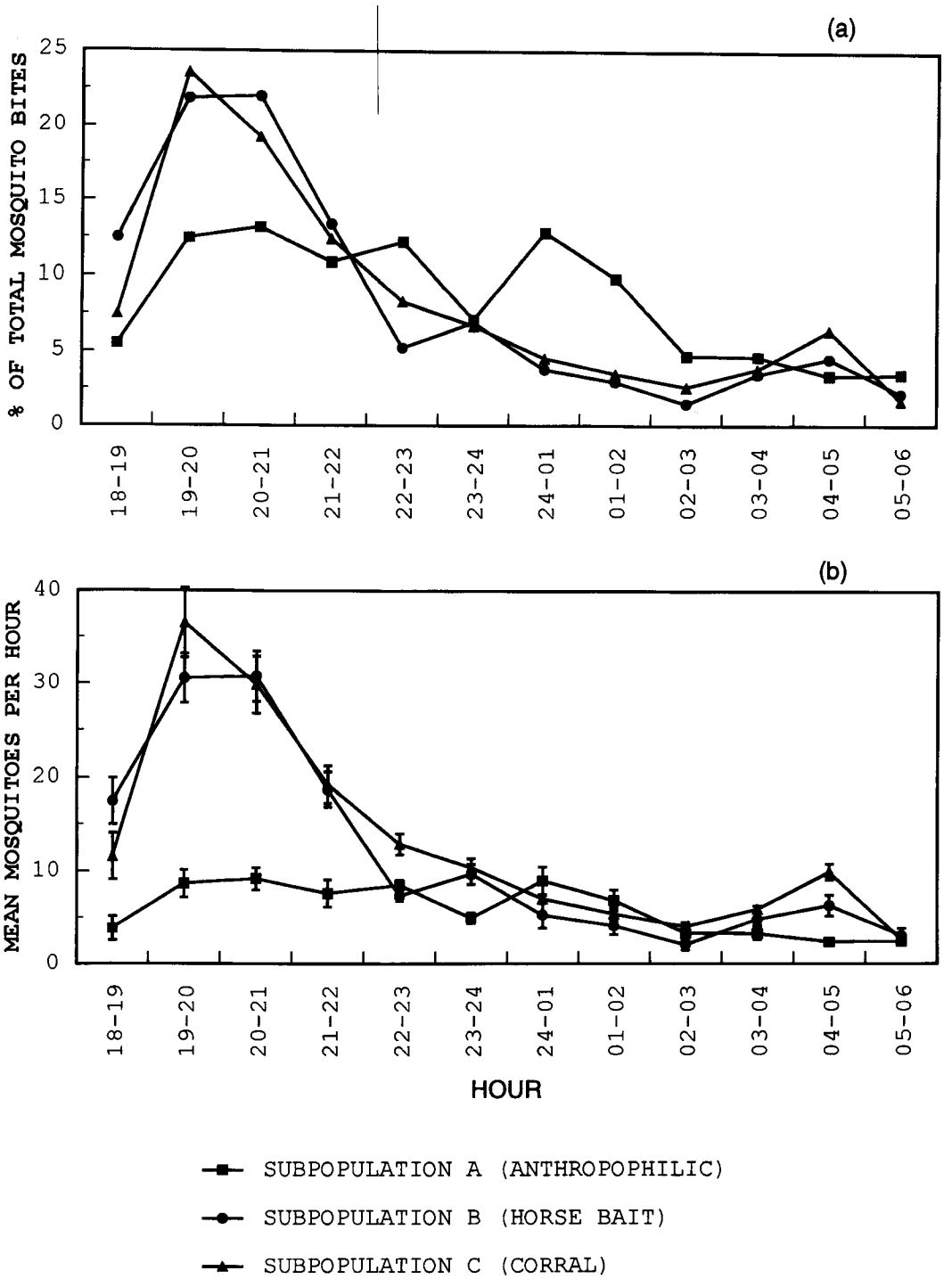

Fig. 2. Biting activity of 3 subpopulations of *Anopheles vestitipennis* in the Lacandón Forest (subpopulations A and B) and Tapachula Coastal Plain (subpopulation C), presented as percent activity (a) and mean (±SE) densities (b).

1.6.4.3.), ACT-1 (E.C. 4.2.1.3), HK-1, HK-2 (E.C. 2.7.1.1.), and AAT-2 (E.C. 2.6.1.1.). Relative migration rates (R_f values) of electromorphs were calculated using the inbred ROCK/DAVIS strain of *Aedes aegypti* (Linn.) as a reference (Munstermann 1988).

Gene frequency data were analyzed using the Byosis-1 program (Swofford and Selander 1981), and similarities and differences among populations were established using Rogers's *I* genetic similarity coefficient (Rogers 1972) and Nei's *D* unbiased genetic distance (Nei 1978).

RESULTS

Biting cycle: A total of 1,220, 1,957, and 1,552 mosquitoes were caught in 20 human landing, 14 horse-baited trap, and 16 corral all-night collections, respectively.

The biting activity in the zoophilic subpopulations B and C of *An. vestitipennis* occurred mostly before midnight (82 and 78%, respectively, for subpopulations B and C), and was bimodal with one peak between 1900 and 2100 h and a second between 0400 and 0500 h, whereas the biting activity of the anthropophilic subpopulation A was fairly constant from 1900 to 0300 h (Fig. 2a). Highest mosquito densities were observed in subpopulation C with a maximum of 36.54 mosquitoes per hour (m/h) (SE, ±3.78) (24% of total bites), between 1900 and 2000 h and a minimum of 2.58 ± 0.05 m/h between 0500 and 0600 h (mean biting = 12.93 ± 3.95 m/h); subpopulation B had maximum and minimum densities at 2000–2100 h (30.75 ± 2.72 m/h; 22% total bites) and 0200–0300 h (2.04 ± 0.58 m/h; 2% total bites), respectively; and subpopulation A had a maximum of 9.17 ± 1.20 m/h at 2000–2100 h (13% total bites), and a minimum of 2.40 ± 0.35 m/h at 0500–0600 h (3% total bites) (Fig. 2b). Differences in mean biting were found among subpopulations in the 2-way factorial analysis ($P = 0.003$), and with time ($P = 0.04$), but no significance was found in the interaction ($P = 0.76$). *Post-hoc* differences were found between subpopulation B vs. subpopulation A ($P = 0.004$) and subpopulation C vs. subpopulation A ($P = 0.04$), but no differences were found between subpopulations B and C ($P = 0.11$).

Electrophoretic studies: Genetic similarity and distance indices were calculated using 13 enzyme loci (Table 1). Eleven of 13 loci were polymorphic (84.6%) with only DIA and HK-1 exhibiting no variation. Moderate heterogeneity was observed with the highest Nei's *D* and lowest Rogers's *I* values found between the 2 sympatric subpopulations A and B ($D = 0.070$, $I = 0.863$), followed by the comparisons between

Table 1. Allele frequencies in the 3 subpopulations of *Anopheles vestitipennis* at 11 of 13 enzyme loci (DIA and HK-1 were monomorphic); *n* = number of mosquitoes studied.

Locus (relative mobility)	Subpopulation		
	A	B	C
IDH-2			
n	21	22	13
0.63	0.000	0.023	0.000
0.84	0.548	0.477	0.538
1.00	0.452	0.500	0.308
1.38	0.000	0.000	0.154
PGM			
n	4	22	3
0.98	1.000	0.977	1.000
1.08	0.000	0.023	0.000
PGI			
n	7	21	11
0.95	0.857	1.000	0.955
1.15	0.143	0.000	0.045
MDH-1			
n	22	22	17
0.63	1.000	1.000	0.971
0.95	0.000	0.000	0.029
ME			
n	22	22	16
0.93	1.000	1.000	0.969
1.13	0.000	0.000	0.031
HAD			
n	16	20	13
0.37	0.031	0.000	0.038
0.56	0.344	0.825	0.192
0.75	0.625	0.175	0.769
6PGD			
n	3	22	7
1.5	1.000	0.977	1.000
1.71	0.000	0.023	0.000
αGPD			
n	9	22	12
1.38	0.677	0.886	1.000
1.62	0.333	0.114	0.000
ACT-1			
n	1	9	3
0.79	0.000	0.722	0.667
1.37	1.000	0.278	0.333
HK-2			
n	22	22	15
1.16	0.000	0.045	0.000
1.25	1.000	0.955	1.000
AAT-2			
n	22	22	12
1.05	0.000	0.023	0.000
1.28	0.136	0.045	0.042

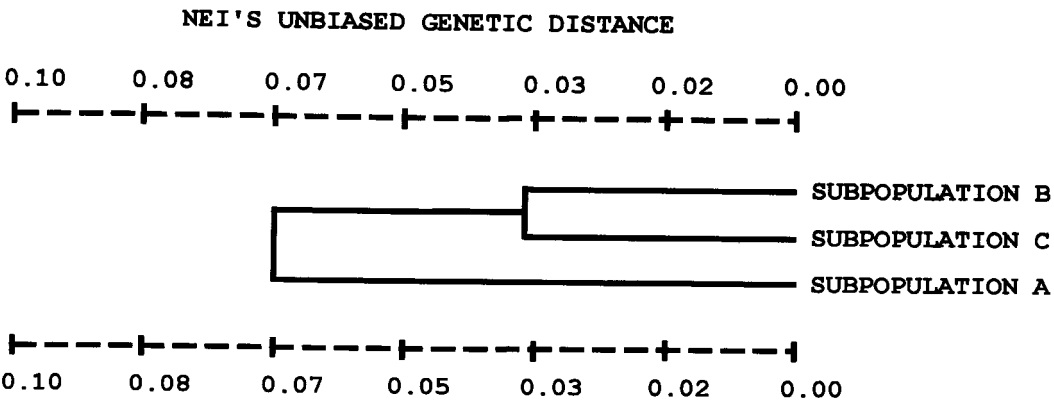

Fig. 3. Cluster diagram of the genetic distance (*D*) among 3 subpopulations of *Anopheles vestitipennis* from southern México.

subpopulations A and C (D = 0.047, I = 0.881) and between subpopulations B and C (D = 0.031, I = 0.909). Differences among subpopulations were largely due to HAD, which provided the greatest impact on these results. A cluster diagram with Nei's genetic distance illustrates the grouping of the 3 subpopulations (Fig. 3).

DISCUSSION

Differences in biting activity of *An. vestitipennis* suggest the existence of 2 behaviorally distinct mosquito ecotypes with sympatric distribution in the Lacandón Forest. The only other allopatric subpopulation tested (collected in a cattle corral in the coastal plain), showed very similar biting patterns to the subpopulation collected using an animal-baited trap in the Lacandón Forest. The anthropophilic segment of the population (A) had a steady biting activity that decreased past midnight, whereas the 2 zoophilic subpopulations (B and C) had the highest biting peaks early at night and just before sunrise. Biting patterns in the middle or late parts of the night have been associated with efficient vectors of malaria, whereas biting patterns with highest peaks early at night are indicative of poor vectors (Hamon 1963, Elliott 1972). Another anthropophilic American malaria vector, *Anopheles darlingi* Root shows wide variation in its biting activity according to geographic areas (Rosa-Freitas et al. 1992), but no differences in its behavior have been reported in the same village and night. Overnight feeding activity results are commonly grouped from various villages (Rubio-Palis and Curtis 1992), masking possible differences that may occur between and within villages. Our results are indicative of 2 distinct subpopulations of *An. vestitipennis* in southern México, but, in order to fully document behavioral differences, further studies are necessary to establish host preferences in these mosquito groups.

Electrophoretic analysis indicated that the differences and similarities found among the subpopulations of *An. vestitipennis* examined in this study may have a genetic basis. The calculated genetic distance between the 2 sympatric subpopulations (0.07), is higher than that between *Anopheles marshallii* (Theobald) species A and species C (0.03) (Lambert 1983), but much lower than the value found in *Anopheles maculipennis* Meigen and *Anopheles sacharovi* Favr (0.66) (Cianchi et al. 1981).

Although the *D* value obtained for *An. vestitipennis* subpopulations A and B could be referred as low (Bullini and Coluzzi 1982, Coluzzi 1988), it suggests that some degree of isolation may be involved in the maintenance of 2 distinct subpopulations and may indicate incipient but identifiable genotypic clusters (Mallet 1995). The smaller genetic distance found between subpopulations B and C may be attributed to the allopatric nature of both subpopulations. During incipient speciation natural selection acts at a faster pace to insure reproductive isolation in sympatric subpopulations following a period of isolation (Mayr 1963). This was further demonstrated by the high relatedness of zoophilic subpopulations B and C, in spite of being geographically isolated.

Vector incrimination studies (Arredondo-Jiménez 1995[3]) indicated that *An. vestitipennis* is not a malaria vector in the coastal plain, but its vectorial role in the Lacandón Forest (Loyola et al. 1991) and its extension into Guatemala have been documented (Padilla et al. 1992). In addition, midgut content analysis has indicated a more anthropophilic feeding pattern (human

blood index [HBI] = 46%) in the Lacandón Forest subpopulation in comparison to the subpopulation from the coastal plain (HBI = 7%) (Arredondo-Jiménez 1995[3]).

Further studies to establish the existence of distinct forms of *An. vestitipennis* are warranted. Such studies may provide diagnostic markers for discriminating the 2 forms.

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