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SHORT COMMUNICATION:

Allozyme differentiation among allopatric populations of *Anopheles nuneztovari* (Diptera: Culicidae)

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ABSTRACT

Four enzymes in six geographic populations, four Brazilian and two Colombian, of *Anopheles nuneztovari* were studied. There were differences among the most frequent alleles in the *EST5*, *ACON* and *MDH* loci in the populations from Brazil and Colombia. The α-*GPD*C* allele was encountered very frequently in the Palo/Sit population in Colombia and the α-*GPD*B* allele was found to be fixed in most of the Brazilian populations. An additional α-*GPD* band was found only in Palo/Sit. The considerable genetic differentiation between populations in western Colombia vs. Brazil suggests that a certain degree of reproductive isolation exists between them.

INTRODUCTION

Anopheles (*Nyssorhynchus*) *nuneztovari* Gabaldón, 1940 occurs in northern South America and can also be found in eastern Panama. This species is considered to be the principal vector of human malaria, particularly for *Plasmodium vivax* in western Venezuela and northwestern Colombia (Kitzmiller *et al.*, 1973; Fajardo and Alzate, 1987). In Brazil, monoclonal studies have shown *A. nuneztovari* to be a potential vector in that it was found infected with *Plasmodium vivax* in the States of Pará (Arruda *et al.*, 1986) and Rondônia (Tadei *et al.*, 1988), and with *P. vivax* and *P. falciparum* in the State of Amapá (Tadei *et al.*, 1993). Infection with *P. vivax* was also registered in eastern Peru (Hayes *et al.*, 1987).

There is evidence for differentiation among geographic populations of *A. nuneztovari*. It may be a complex of cryptic species separable by behavioral (Elliott, 1972), chromosomal (Kitzmiller *et al.*, 1973; Conn *et al.*, 1993) and enzymatic characteristics (Steiner *et al.*, 1980). On the other hand, analyses of the ITS2 region of the ribosomal DNA of nine distinct populations showed minimal differences, that were not considered sufficient to propose a species complex for *A. nuneztovari* (Fritz *et al.*, 1994). Similar results were obtained with mitochondrial DNA (Conn *et al.*, unpublished results). These data could be better understood with measurements of the reproductive isolation between the populations by crossing experiments in the laboratory. However, the species that constitute the subgenus *Nyssorhynchus*, in general, do not reproduce in the laboratory. We report loci that permit differentiation among populations in Brazil and Colombia, based on allozyme studies.

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MATERIAL AND METHODS

Four populations in Brazil and two in Colombia were studied, namely: Km 206 of the BR-174 highway - 1°16'S, 60°23'W (AM), Puraquequara - 3°6'7"S, 60°1'30"W (AM), Tucuruí - 3°42'S, 49°27'W (PA) and Nova Mazagão - 0°7'S, 51°17'W (AP); Tibú - 8°39'N, 72°42'W (north of Santander - northern Colombia), Palo Grande Calle Larga and Sitronela - Palo/Sit - 3°49'N, 77°4'W (Valle - western Colombia). For the analysis, descendants of females captured in nature were used. An average of two to four individuals were used from each batch. The following systems were studied: esterase (EST), aconitase (ACON) and malate dehydrogenase (MDH) in 4th stage larvae and α-glycerophosphate dehydrogenase (α-GPD) in recently emerged adults. Electrophoresis was carried out in horizontal starch gels (12%). The methods for the analysis of EST and α-GPD are described in Scarpassa (1988). The CA-7 buffer system was used for ACON (Steiner and Joslyn, 1979) and in the development of the isozymes we used the methodology of Harris and Hopkinson (1976). For the analysis of MDH we used the phosphate-citrate buffer (0.245 M in sodium phosphate and 0.15 M in citric acid), pH 5.9, for the electrodes, dilution 1:40 in gel. For the reaction mixture we used the methodology of Falcão (1984). The identifications were based on Sutil (1976).

RESULTS AND DISCUSSION

For the esterases, five activity regions were observed in the six populations, and *EST5* was analyzed (Figure 1). This region presents the best resolutions and gives the most credibility to the data. Elevated variability was observed in the *EST5* locus in the four Brazilian populations (Table I). Five codominant alleles were observed: *EST5*A*, *EST5*B*, *EST5*C*, *EST5*D* and *EST5*E*. The most frequent alleles were *EST5*D* for the BR-174 and Puraquequara populations; *EST5*C* and *EST5*D* for Tucuruí and *EST5*B* and *EST5*D* for Nova Mazagão. However, for the Colombia populations this locus presented low variability and the *EST5*B* and *EST5*C* alleles were detected, the latter with a high frequency in Tibú and Palo/Sit populations. The contrary was observed in Nova Mazagão where this locus showed high variation and the *EST5*C* allele occurred at a low frequency (Table I).

Only one activity region was observed for ACON (Figure 2), suggesting that this enzyme is genetically controlled by only one locus *ACON*, with

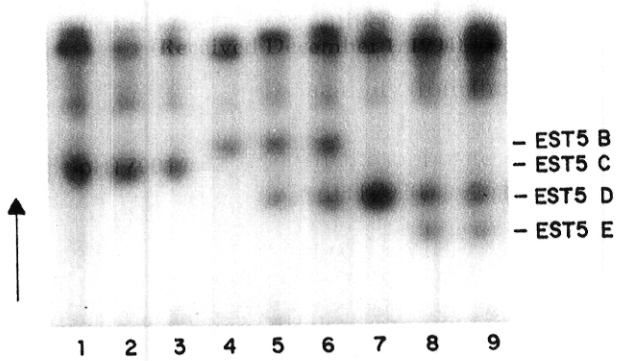

Figure 1 - Isozyme patterns of esterases in *Anopheles nuneztovari* populations, showing the low variability in the *EST5* locus of Palo/Sit (individuals: 1 to 3) and elevated variability in Nova Mazagão (individuals: 4 to 9). Phenotypes - individuals 1, 2 and 3: EST5 C; individual 4: EST5 B; individuals 5 and 6: EST5 BD; individual 7: EST5 D; individuals 8 and 9: EST5 DE.

Table I - Frequencies of alleles at four loci in six geographic populations of *Anopheles nuneztovari*.

Loci		Populations					
		BR-174	PUR	TUC	NOMA	TIBÚ	PALO/SIT
EST5	N	155	128	41	68	78	80
*EST5*A*		0.019	0.008	0.037	0.074	0.000	0.000
*EST5*B*		0.106	0.180	0.061	0.368	0.058	0.019
*EST5*C*		0.248	0.234	0.341	0.110	0.942	0.981
*EST5*D*		0.610	0.516	0.463	0.367	0.000	0.000
*EST5*E*		0.016	0.063	0.098	0.081	0.000	0.000
ACON	N	124	124	164	51	64	60
*ACON*A*		0.000	0.000	0.000	0.000	0.102	0.275
*ACON*B*		0.024	0.004	0.018	0.108	0.156	0.042
*ACON*C*		0.952	0.960	0.979	0.892	0.742	0.667
*ACON*D*		0.024	0.036	0.003	0.000	0.000	0.017
MDH	N	126	152	96	71	72	73
*MDH*A*		0.000	0.026	0.000	0.014	0.000	0.000
*MDH*B*		0.996	0.947	0.964	0.986	0.319	0.322
*MDH*C*		0.004	0.026	0.036	0.000	0.681	0.678
*MDH*D*		0.000	0.000	0.000	0.000	0.000	0.000
α-GPD	N	137	66	135	75	44	97
*α-GPD*A*		0.000	0.000	0.033	0.000	0.000	0.000
*α-GPD*B*		1.000	1.000	0.967	1.000	0.989	0.052
*α-GPD*C*		0.000	0.000	0.000	0.000	0.011	0.948

N = Number of individuals analyzed; BR-174 = BR-174; PUR = Puraquequara; TUC = Tucuruí; NOMA = Nova Mazagão; TIBÚ = Tibú; PALO/SIT = Palo Grande/Sitronela.

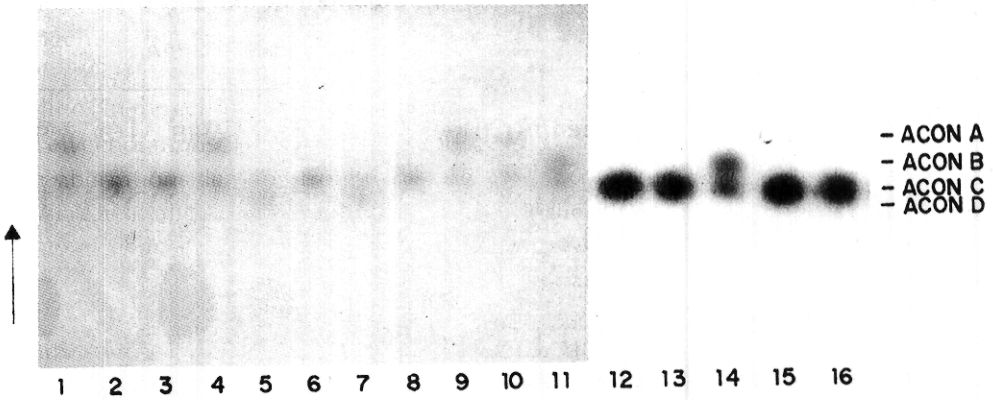

Figure 2 - Isozyme pattern of aconitase in *Anopheles nuneztovari* populations showing the segregation of the four alleles at the *ACON* locus in populations of Palo/Sit (individuals: 1 to 11) and Tucuruí (individuals: 12 to 16). Phenotypes - individuals 1,4,9 and 10: ACON AC; individuals 2,3,6,8,12,13,15 and 16: ACON C; individuals 5 and 7: ACON CD; individuals 11 and 14: ACON BC.

four alleles (Table I). In the Brazilian populations this locus proved not to be very variable. The *ACON*C* allele was the most frequent and the *ACON*B* and *ACON*D* rare. High variability was detected in Tibú and Palo/Sit. Three alleles, *ACON*A*, *ACON*B* and *ACON*C* were detected in Tibú. The last two were the most frequent. In Palo/Sit four alelles were detected: *ACON*A*, *ACON*B*, *ACON*C* and *ACON*D*. *ACON*A* and *ACON*C* were the most frequent (Table I).

One region was visualized for MDH (Figure 3), suggesting that its synthesis can be genetically controlled by an *MDH* locus. This locus proved not to be very variable in the Brazilian populations, and the allele *MDH*B* was fixed in practically all of them (Table I). However, for the Tibú and Palo/Sit populations the *MDH*C* allele was the most frequent, and there was a high number of heterozygote individuals for the *MDH*B/MDH*C* genotype.

Two distinct electrophoretic patterns were observed for α-GPD (Figure 4). The first detected in the Brazilian populations and in Tibú (Colombia) showed only one α-*GPD* locus, monomorphic for the α-*GPD*B* allele in the BR-174, Puraquequara and Nova Mazagão populations. In the Tucuruí and Tibú populations, other than this allele, we also observed the rare alleles α-*GPD*A* and α-*GPD*C* (Table I).

The second pattern was found only in Palo/Sit (Colombia) where the alelle α-*GPD*C* demonstrated a high frequency (Table I). An additional band was also observed in a more cathodic position in relation to the α-*GPD*C* allele present in larvae, pupae and adults. This genic expression pattern differs from the Brazilian populations (Scarpassa and Tadei, 1993). This extra band can be attributed to a possible product of secondary modifications (post-synthesis) of the α-*GPD* locus.

These results give evidence of a greater genetic differentiation among the Brazilian and Colombian populations, especially the Palo/Sit population, which was the most differentiated as regards the α-*GPD* locus. In support of this differentiation, we can cite the behavioral and polytene chromosome studies carried out on these populations. The Brazilian populations are predominantly outdoor biters and are zoophilic (Fleming, cited in Elliott, 1972; Tadei, personal communication). The populations in western Venezuela and northwestern Colombia show accentuated anthrophilism, with indoor activity (Elliott, 1972; Fajardo and Alzate, 1987). The mosquitoes analyzed in this paper were collected under these same conditions, that is, in Brazil the specimens were captured predominantly in exophilism and zoophilism conditions. In Colombia

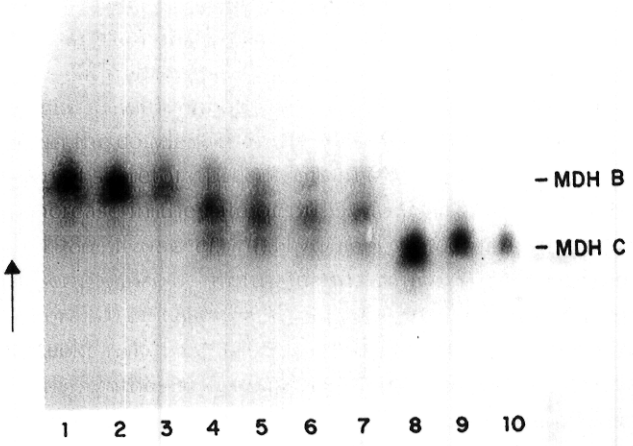

Figure 3 - Isozyme pattern of malate dehydrogenase in *Anopheles nuneztovari*, showing the allele *MDH*B* in the Tucuruí population (individuals 1 to 3) and the alleles *MDH*B* and *MDH*C* in the Palo/Sit population (individuals 4 to 10). Phenotypes - individuals 1,2 and 3: MDH B; individuals 4,5,6 and 7: MDH BC; individuals 8,9 and 10: MDH C.

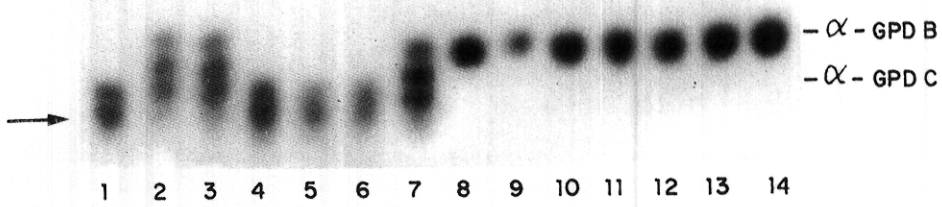

Figure 4 - Isozyme patterns of α-GPD in *Anopheles nuneztovari* in the Palo/Sit populations (individuals 1 to 7) and BR-174 (individuals 8 to 14). Phenotypes - individuals 1,4,5 and 6: α-GPD C; individuals 2,3 and 7: α-GPD BC; individuals 8 to 14: α-GPD B. The arrow points to the additional band.

sampling was indoors and carried out with human bite. Coluzzi (1970) mentions that ecological and ethological differences are generally the first to be detected between members of the species complexes. Chromosome analysis in populations from Sitronela and Sabaleta (western Colombia) showed a very evident chromocenter and two small inversions (2Lc and 2Ld), included in the 2Lb inversion. These rearrangements were not detected in the Brazilian populations (Kitzmiller *et al.*, 1973; Conn *et al.*, 1993). Isozyme studies in populations from Brokopondo (Suriname) and Barinas (Venezuela) showed little differentiation. Only one locus (*EST5*) showed differences among the 23 analyzed. A greater number of individuals and populations need to be studied (Steiner *et al.*, 1980).

Considering the genetic differentiation found between the Palo/Sit (west of the Andes) and Brazilian (east of the Andes) populations, a certain degree of reproductive isolation between the populations can be said to exist. Unfortunately, the difficulty in obtaining reproduction in the laboratory for this species does not permit an evaluation of the reproductive isolation between them. However, these results should be compared to those in the literature, especially with respect to the ribosomal genes (Fritz *et al.*, 1994) and the mitochondrial DNA (Conn *et al.*, unpublished results) in order to permit a better evaluation of the different genetic analyses.

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RESUMO

Foram estudadas quatro enzimas em seis populações geográficas de *Anopheles nuneztovari*, sendo quatro do Brasil e duas da Colômbia. Os dados evidenciaram diferenças quanto aos alelos mais frequentes nos locos *EST5*, *ACON* e *MDH*, entre populações do Brasil e da Colômbia. Considerando o loco α-*GPD*, observou-se o alelo α-*GPD*C* em freqüências elevadas na população de Palo/Sit e o alelo α-*GPD*B* fixado na maioria das populações brasileiras. Ainda para α-GPD, observou-se a presença de uma banda adicional que foi exclusiva de Palo/Sit. Estes resultados evidenciam maior diferenciação genética entre populações do oeste da Colômbia e do Brasil, podendo-se predizer a existência de algum grau de isolamento reprodutivo entre elas.

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