ENZYMATIC ANALYSIS IN *Anopheles nuneztovari* GABALDÓN (DIPTERA, CULICIDAE)

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ABSTRACT

Enzymatic analysis in *Anopheles nuneztovari* was made using four populations from the Brazilian Amazon and two from Colombia. The enzymes ME and XDH presented a monomorphic locus in all of the studied populations. EST and LAP presented a higher number of loci. In EST, genetic variation was observed in the five loci; LAP presented four loci, with allec variation in two loci. In IDH, three activity regions were stained, with genetic variation for locus *Idh-1* in the Brazilian Amazon populations. A locus for MDH was observed, with genetic variation in the six populations. A region was verified for ACON, with four alleles in Sitronela and three in the other populations. PGM constituted one locus, with a high variability in the Brazilian Amazon populations. A locus was observed for 6-PGD with allelic variation in all of the populations with the exception of Tibú. Enzyme PGI presented two loci, both with genetic variability in the Tucuruí population. The enzyme α-GPD showed an activity region with polymorphism in the Tucuruí, Tibú and Sitronela populations. The phenotypic variations detected for these enzymes suggest that four (EST, LAP, ACON and PGM) possess monomeric structures and five (IDH, MDH, 6-PGD, PGI and α-GPD) dimeric structures in their proteins. These enzymes constitute in important markers to estimate variability and genetic divergence in natural populations of *A. nuneztovari.*

Key words: isozymes, electrophoretic profiles, genetic variation, neotropical anopheline, Amazonian.

RESUMO

Análise enzimática em *Anopheles nuneztovari* Gabaldón (Diptera, Culicidae)

Foi realizada análise enzimática em *Anopheles nuneztovari* em quatro populações da Amazônia, Brasil, e em duas da Colômbia. As enzimas ME e XDH mostraram um loco monomórfico em todas as populações estudadas. A EST e a LAP apresentaram maior número de locos. Na primeira, observou-se variação genética nos cinco locos revelados; na segunda dos quatro locos, dois apresentaram variação alélica. Na IDH, três regiões de atividade foram reveladas, com variação genética para o loco *Idh-1* em populações da Amazônia. Observou-se um loco para a MDH, com variação nas seis populações. Uma região foi verificada para ACON, com quatro alelos na população de Sitronela e três nas demais populações. A PGM consistiu de um loco, com variabilidade elevada nas populações da Amazônia. Um loco foi verificado para 6-PGD com variação alélica em todas as populações, exceto em Tibú. A enzima PGI apresentou dois locos, ambos com variação apenas na população de Tucuruí. A α-GPD consistiu de uma região de atividade, com variação nas populações de Tucuruí, Tibú e Sitronela. A variação fenotípica detectada para estas enzimas sugere que quatro (EST, LAP,

ACON e PGM) possuem estrutura monomérica e cinco (IDH, MDH, 6-PGD, PGI e α-GPD), estrutura dimérica em suas proteínas. Essas enzimas constituem-se em importantes marcadores para estimar variabilidade e divergência genética em populações naturais de *A. nuneztovari*.

Palavras-chave: isoenzimas, perfis eletroforéticos, variação genética, anofelino neotropical, Amazônia.

INTRODUCTION

In *Anopheles*, *Aedes* and *Culex* geni, isozymes have been used in genetic variability analysis among populations within species, and also as genetic markers important in the sibling species separation, by diagnostic loci or by a combination of several loci that show differentiation (Narang & Seawright, 1990). In anopheline mosquitoes, diagnostic loci are frequently used to separate morphologically similar species in several species complexes. The identification of these species becomes relevant, as it allows separation of members that are involved in human malaria transmission (Coluzzi, 1988). Thus, interpretation of electrophoretic profiles of different enzymatic systems is a basic necessity for these studies.

In this study, we interpreted the electrophoretic profiles of eleven enzymes in six populations of the human malaria vector *Anopheles (Nyssorhynchus) nuneztovari*, including the photos for each enzyme, the number of activity zones coded by different loci and their possible allelic variants. Population structure and genetic divergence among these populations were analyzed by Scarpassa *et al*. (1999).

MATERIAL AND METHODS

Samples of *A. nuneztovari* were collected at four sites in the Brazilian Amazon [km 206 of the BR-174 Highway (BR), Amazonas State (1º16'S, 60º23'W); Puraquequara (PUR), Amazonas State (3º6'7''S, 60º1'30''W); Tucuruí (TUC), Pará State (3º42'S, 49º27'W); and Nova Mazagão (NOMA), Amapá State (0º7'S, 51º17'W)], and two sites in Colombia [Tibú (TIBÚ), Norte de Santander Department (8º39'N, 72º42'W); and Sitronela (SIT), Valle Department (3º49'N, 77º4'W)]. The specimens were captured when feeding on pigs, cattle, resting on stable walls or human biting. After captures, blood-fed mosquitoes were individually isolated in plastic cups for egg laying, according to Scarpassa & Tadei (1990). Following oviposition and eclosion, the offspring (F_1) were reared until the 4th instar larvae, pupae and adults, when they were frozen at –70°C, until analyzed. Fourth instar larvae were used in the analysis of ten enzymes, except for α-glycerophosphate dehydrogenase (α-GPD), for which adults were used. An average of 2-4 individuals from each progeny were employed. Electrophoresis was carried out in starch (12%) and starch-agarose gels (1% and 0.8%, respectively). Buffer solutions and reactions mixtures were according to Harris & Hopkinson (1976), Steiner & Joslyn (1979) and Scarpassa *et al*. (1999).

The electrophoretic conditions used for each enzyme are showed in the Table 1. Identification of the specimens were done on eggs, adults and male genitalia (Cova-Garcia, 1961; Gorham *et al*., 1967; Savage, 1986). The denomination employed for loci and alleles was according to Manguin *et al*. (1995) and Scarpassa *et al*. (1999), frequently used in mosquitos, where the allele most common was considered as 100.

RESULTS AND DISCUSSION

The electrophoretic patterns of the eleven enzymatic systems were analyzed: malic enzyme (ME, E.C.1.1.1.40), xanthine dehydrogenase (XDH, E.C.1.2.1.37), esterase (EST, E.C.3.1.1.1), leucine aminopeptidase (LAP, E.C.3.4.11.1), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), malate dehydrogenase (MDH, E.C.1.1.1.37), aconitase (ACON, E.C.4.2.1.3), 6 phosphogluconate dehydrogenase (6-PGD, E.C.1.1.1.44), phosphoglucomutase (PGM, E.C.5.4.2.2), phosphoglucose isomerase (PGI, E.C.5.3.1.9) and α-glycerophosphate dehydrogenase (α-GPD, E.C.1.1.1.8). The number of involved loci and their allelic variants of each enzyme are described below.

Malic Enzyme and Xanthine Dehydrogenase

The electrophoretic profiles of the ME and XDH enzymes presented one activity zone and they were monomorphic in the studied populations (Figs. 1A and B, Table 2).

TABLE 1

Electrophoretic conditions used in the eleven enzymes of *Anopheles nuneztovari*.

Enzymes	Buffer		Gel type	Migration time (hours)	V/cm
	Bridge	Gel			
EST, LAP	0.3 M Borate pH 8.0	0.17-0.0023 M Tris-citrate pH 8.0	Starch	12	1.7
IDH, ME, MDH	0.245-0.15 M Phosphate-citrate pH 5.9	1:40 dilution (v:v) of the bridge buffer	Starch	15	2.6
ACON, 6-PGD	0.135-0.040 M Tris-citrate pH 6.90	0.009-0.003 M Tris-citrate pH 7.10	Starch	16	2.3
PGM	0.22 M TEMM pH 7.4	1:15 dilution (v:v) of the bridge buffer	Starch-agarose	5	4.8
PGI	0.22 M TEMM pH 7.4	1:15 dilution (v:v) of the bridge buffer	Starch	16	2.3
α-GPD	0.1 M Tris-phosphate pH 7.4	1:20 dilution (v:v) of the bridge buffer	Starch	16	2.6
XDH	0.036-0.194 M Lithium-borate pH 8.25	0.074-0.009 M Tris-citrate pH 8.45	Starch	15	1.7

Similar results were obtained in several species of the *Nyssorhynchus* subgenus (Narang *et al*., 1979; Hii *et al*., 1991; Narang *et al*., 1991).

For ME, however, one locus with two or more alleles was verified in the four species of *A. quadrimaculatus* complex (Narang *et al*., 1989), in *A. deaneorum* and *A. marajoara* (Narang *et al*., 1993) and in *A. pseudopunctipennis* (Manguin *et al*., 1995). Two loci with genetic variation were found in the *A. punctulatus* complex (Foley *et al.*, 1995). Genetic variation was detected for XDH in at least one locus in *A. aquasalis* (Steiner *et al*., 1981), in the *A. quadrimaculatus* complex (Narang *et al*., 1989), in *A. albimanus* (Narang *et al*., 1991), and in *A. deaneorum* and *A. marajoara* (Narang *et al*., 1993).

Esterase

Analysis of the 4th instar larvae presented an electrophoretic profile complex with five activity zones in the six populations. The allelic variants observed for all of these isozymes allow for the proposal that they are coded by independent loci: *Est-1*, *Est-2*, *Est-3*, *Est-4* and *Est-5*. In the *Est-1* to *Est-4* loci the number of alleles present cannot be quantified, due to overlapping of the alleles among these loci. Detailed analysis was possible only on the *Est-5* locus (Fig. 1C). In the four Brazilian Amazon populations, five codominant alleles were observed, being the most frequents $Est\text{-}5_{109}$, $Est\text{-}5_{106}$ and $Est\text{-}5_{100}$ (Table 2). The population from NOMA presented the highest number of genotype combinations, with 12 phenotypes. The two Colombian populations presented only two alleles $Est\text{-}5_{109}$ and $Est\text{-}5_{106}$. The $Est\text{-}5_{106}$ allele showed high frequency in both populations. The heterozygous individuals presented two bands, suggesting a monomeric structure for this isozyme.

Our data support previous studies, which indicate that esterase is the most variable enzyme in mosquitoes, as well as in other insects, small vertebrates and plants (Wagner & Selander, 1974). In anopheline mosquitoes such as in *A. albimanus* of the six stained loci, four were polymorphic (Vedbrat & Whitt, 1975), in *A. aquasalis*, five of the six stained loci presented genetic variation (Narang *et al*., 1979), in *A. nuneztovari* from Suriname and

Venezuela, three of the five loci presented variation (Steiner *et al*., 1980), as well as three of the six loci in *A. darlingi* (Santos *et al*., 1996), and two of the four loci in *A. albitarsis* (Maia, 1997).

TABLE 2

Frequencies of alleles at sixteen loci of *Anopheles nuneztovari*.

		Population					
Locus	**Allele**	**BR**	**PUR**	**TUC**	**NOMA**	**TIBÚ**	**SIT**
Pgm	n	141	158	52	136	85	85
	113	0.039	0.013	0	0.026	0	0
	108	0.429	0.516	0.019	0.371	0.265	0.412
	100	0.362	0.263	0.875	0.445	0.735	0.588
	94	0.149	0.196	0.096	0.011	0	0
	91	0.021	0.013	0.010	0.121	0	0
	89	0	0	0	0.026	0	0
6Pgd	n	130	124	89	67	64	62
	108	0	0.012	0	0.007	0	0
	100	0.988	0.988	0.983	0.985	1	0.952
	92	0.012	0	0.017	0.007	0	0.048
Acon	n	112	124	76	51	64	60
	106	0	0	0	0	0.102	0.275
	103	0	0.004	0	0.108	0.156	0.042
	100	0.991	0.960	1	0.892	0.742	0.667
	98	0.009	0.036	0	0	0	0.017
Mdh	n	104	152	99	71	72	73
	113	0	0.026	0.005	0.014	0	0
	100	1	0.947	0.949	0.986	0.319	0.322
	94	0	0.026	0.035	0	0.681	0.678
	78	0	0	0.010	0	0	0
Idh-1	n	138	169	99	83	66	54
	106	0.029	0.012	0.030	0.102	0	0

TABLE 2 (*Continued*)

		Population					
Locus	**Allele**	**BR**	**PUR**	**TUC**	**NOMA**	**TIBÚ**	**SIT**
	100	0.964	0.988	0.934	0.898	1	1
	93	0.007	0	0.035	0	0	0
αGpd	n	55	66	56	75	44	97
	107	0	0	0.036	0	0	0
	100	1	1	0.964	1	0.989	0.052
	90	0	0	0	0	0.011	0.948
Lap-1	n	156	145	130	80	31	69
	100	1	0.976	1	1	1	1
	98	0	0.024	0	0	0	0
Lap-5	n	160	147	21	120	87	72
	100	1	1	0.857	0.967	0.937	0.938
	98	0	0	0.143	0.033	0.063	0.063
Est-5	n	142	128	100	68	78	80
	111	0.007	0.008	0.025	0.074	0	0
	109	0.190	0.180	0.330	0.368	0.058	0.019
	106	0.239	0.234	0.485	0.110	0.942	0.981
	100	0.532	0.516	0.145	0.367	0	0
	97	0.032	0.063	0.015	0.081	0	0
Pgi-1	n	73	123	28	75	69	69
	100	1	1	0.911	1	1	1
	96	0	0	0.089	0	0	0
Pgi-2	n	65	78	84	75	69	69
	110	0	0	0.024	0	0	0

TABLE 2 (*Continued*)

		Population					
Locus	**Allele**	**BR**	**PUR**	**TUC**	**NOMA**	**TIBÚ**	**SIT**
	100	1	1	0.952	1	1	1
	93	0	0	0.024	0	0	0
Lap-2	n	161	151	181	125	102	86
	100	1	1	1	1	1	1
Lap-4	n	159	148	182	126	103	85
	100	1	1	1	1	1	1
Idh-2	n	110	130	72	68	66	54
	100	1	1	1	1	1	1
Me	n	139	154	56	68	60	60
	100	1	1	1	1	1	1
Xdh	n	112	104	45	52	48	54
	100	1	1	1	1	1	1

BR = BR-174 Highway; PUR = Puraquequara; TUC = Tucuruí; NOMA = Nova Mazagão; TIBÚ = Tibú; and SIT = Sitronela.

Also, ontogenetic analysis of the *A. nunez-tovari* population from Tucuruí, presented seven loci with genetic variation in six (Scarpassa, 1988). These results suggest that esterase isozymes are important markers for estimating genetic variability in natural populations of *anopheline mosquitoes*.

Leucine Aminopeptidase

Leucine aminopeptidase showed four electronegative activity zones in the six populations. *Lap-1* and *Lap-5* presented a weak coloration, and *Lap-2* and *Lap-4* were intensely colored (Fig. 1D). In this study, *Lap-3* was not visualized because it is exclusive for pupa and adult stages (Scarpassa *et al.*, 1992). The *Lap-1* locus was monomorphic in all populations, except in the population from PUR, which showed two alleles, $Lap\text{-}1_{100}$ and $Lap\text{-}1_{98}$ (Table 2). *Lap-2* and *Lap-4* loci were monomorphic in all the populations. The *Lap-5* locus showed two codominant alleles, being the $Lap\text{-}5_{100}$ allele fixed in the PUR, and it was more common in the populations from BR, TUC, NOMA, TIBÚ and SIT (Table 2). Two bands in the heterozygous individuals were verified, which allows to propose that the protein structure is monomeric. We also observed in the *Lap-5* locus two additional bands in heterozygous individuals and exclusive of the population from TUC. This results suggests that the presence of these bands may be a consequence of post-translational changes. However, this hypothesis may be reinforced with additional studies.

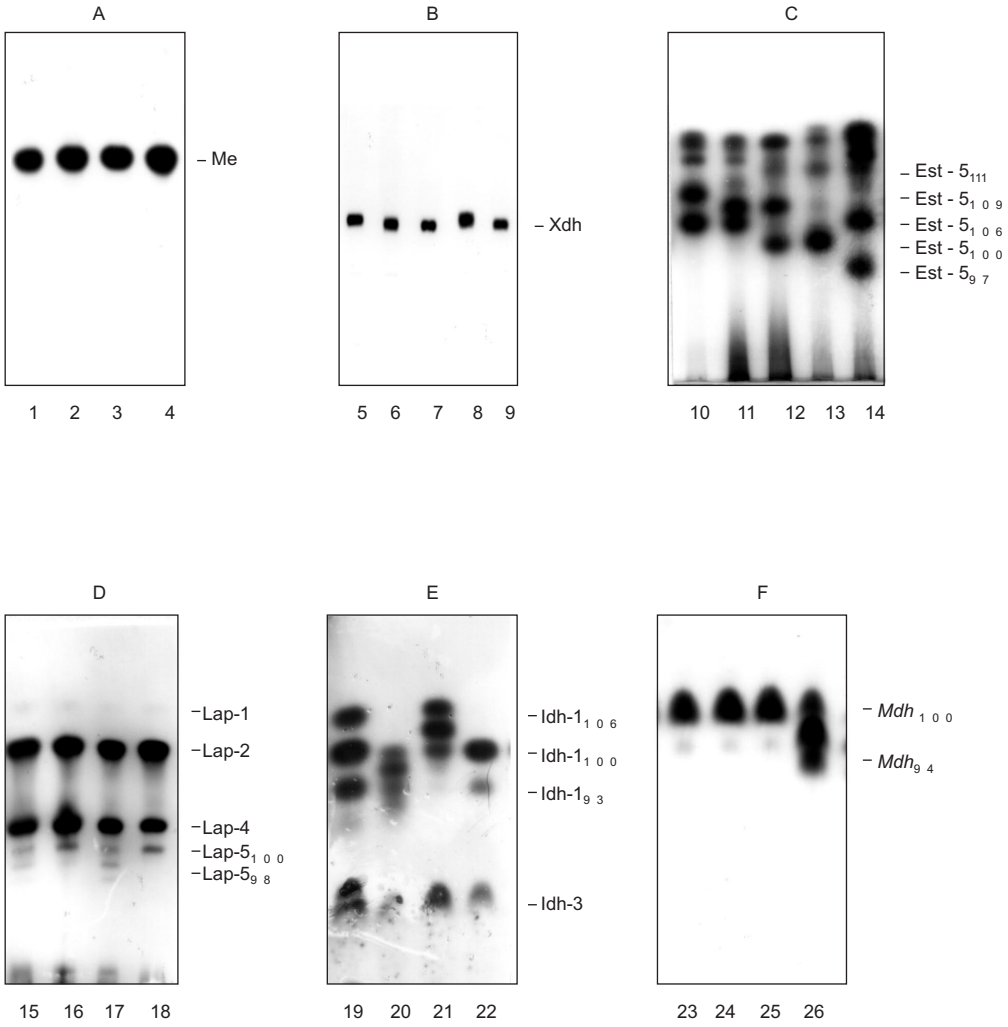

Fig. 1 — *Anopheles nuneztovari*. **A)** Malic enzyme. Samples from PUR. **B)** Xanthine dehydrogenase. Samples from PUR. **C)** Esterase. Samples from TUC (10, 11) and BR (12 to 14). Phenotypes of the *Est-5* locus: *Est-5*$_{111/106}$ (10), *Est*-5$_{109/106}$ (11), *Est-5*$_{109/100}$ (12), *Est-5*$_{100}$ (13), *Est-5*$_{106/97}$ (14). **D)** Leucine aminopeptidase. Samples from TUC. Phenotypes of the *Lap-5* locus: *Lap-5* $_{100/98}$ (15, 17); *Lap-5*$_{100}$ (16, 18). **E)** Isocitrate dehydrogenase. Samples from TUC. Phenotypes of the *Idh-1* locus: *Idh-1*$_{106/93}$ (19), *Idh-1*$_{100/93}$ (20), *Idh-1*$_{106/100}$ (21), *Idh-1*$_{100}$ (22). **F)** Malate dehydrogenase. Samples from TUC. Phenotypes of the *Mdh* locus: *Mdh*$_{100}$ (23 to 25), *Mdh*$_{100/94}$ (26).

Low variability for this enzyme in *A. nuneztovari*, is in accordance with results found for others species of Neotropical anopheline, such as *A. aquasalis* (Narang *et al*., 1979), *A. darlingi* (Santos *et al*., 1996), *A. oswaldoi* (Scarpassa, V. N. 2, unpubl. data), among others. Agreeing with the above results, ontogenetic analysis in *A. nuneztovari* showed six loci with variation in only one (Scarpassa *et al*., 1992).

Isocitrate Dehydrogenase

Isocitrate dehydrogenase showed three electronegative activity zones in the six populations (Fig. 1E). *Idh-1* presented allelic variation in the four Brazilian Amazon populations. This suggests that it is genetically controlled by the *Idh-1* locus, with three codominant alleles: *Idh-1*$_{106}$, *Idh-1*$_{100}$ and *Idh-1*$_{93}$. The *Idh-1*$_{100}$ allele was the most com-

mon in the Brazilian Amazon (Table 2). The combination of these alleles resulted in the following phenotypes: *Idh-1*$_{106/100}$ and *Idh-1*$_{100}$, detected in the populations from PUR and NOMA; *Idh-1*$_{106/100}$, *Idh-1*$_{100}$ and *Idh-1*$_{100/93}$, in the population from BR; and *Idh-1*$_{106/100}$, *Idh-1*$_{106/93}$, *Idh-1*$_{100}$ and *Idh-1*$_{100/93}$, in the population from TUC.

In the Colombian populations the *Idh-1*$_{100}$ allele was fixed. *Idh-2* presented a weak intensity band, probably determined by one monomorphic locus in the six populations. Three bands in the heterozygous individuals suggest a dimeric protein structure. *Idh-3*, despite showing a strong intensity in coloration, included variable bands in its electrophoretic mobility. It was not possible to interpret the genetic mechanism involved for this zone.

Comparing these results with those obtained for other anophelines, it can be verified that only two loci have been described, with allelic variation occurring in at least one. Examples of this can be seen in *A. stephensi* (Van Driel *et al.*, 1987), *A. quadrimaculatus* complex (Narang *et al*., 1989), *A. albimanus* (Narang *et al*., 1991), *A. balabacensis* (Hii *et al*., 1991), *A. dirus* complex (Green *et al*., 1992), *A. darlingi* (Santos *et al*., 1996). However, the *Idh-3* observed in *A. nuneztovari* is probably a third locus detected for the first time in Neotropical anophelines.

Malate Dehydrogenase

Malate dehydrogenase showed an electronegative zone in all studied populations (Fig. 1F). This zone presented four codominant alleles: *Mdh*$_{113}$, *Mdh*$_{100}$, *Mdh*$_{94}$ and *Mdh*$_{78}$. The *Mdh*$_{100}$ allele had frequencies over 94% in the populations from the Brazilian Amazon, while the *Mdh*$_{113}$, and *Mdh*$_{78}$ alleles were observed low frequencies in the PUR, TUC and NOMA populations. On the other hand, the *Mdh*$_{94}$ allele was the most frequent in the populations from TIBÚ and SIT (Table 2). The heterozygous individuals presented three bands, suggesting a dimeric enzyme structure. During analysis a less anodic region was observed. Its intensity varied according to the activity of the *Mdh* locus´s bands. We believe that the presence of this region may be due to an isozyme of secondary origin.

Similarly to *A. nuneztovari*, one or two loci have been revealed with genetic variation in at least one, as in the *A. quadrimaculatus* complex (Narang *et al*., 1989), *A. minimus* (Green *et al*., 1990), *A. albimanus* (Narang *et al*., 1991), *A. balabacensis* (Hii *et al*., 1991), *A. pseudopunctipennis* (Estrada-Franco *et al*., 1993; Manguin *et al*., 1995), and *A. punctulatus* complex (Foley *et al*., 1995), among others.

Aconitase

A single activity zone of aconitase was detected in the six studied populations (Fig. 2A). In the Brazilian Amazon populations, the *Acon*$_{100}$ allele was the most common, and *Acon*$_{103}$ and *Acon*$_{98}$ alleles were rare (Table 2). Elevated variation was verified in the SIT populatoin with four alleles: *Acon*$_{106}$, *Acon*$_{103}$, *Acon*$_{100}$ and *Acon*$_{98}$. In SIT the *Acon*$_{106}$ and *Acon*$_{100}$ alleles were the most frequent; in TIBÚ were detected the first three. *Acon*$_{103}$ and *Acon*$_{100}$ were the most common. The heterozygous individuals showed two bands, indicating that the protein may be monomeric in structure.

However, Fritz *et al*. (1995) found two loci for Aconitase in *A. nuneztovari* from Venezuela, using adults. It is possible that the *Acon-1* locus, detected by these authors, is the locus *Acon* in the present study. The differences between these two studies may be due to distinct ontogenetic patterns. In other anophelines, where adult individuals were analyzed, two loci were also revealed with genetic variation for the *Acon-1* locus, such as in the *A. punctulatus* complex (Foley *et al*., 1995) and in *A. rangeli* and *A. trinkae* (Fritz *et al*., 1995).

6-Phosphogluconate Dehydrogenase

6-Phosphogluconate dehydrogenase presented one zone, with bands of strong intensity (Fig. 2B). In populations from BR, TUC and NOMA the *6-Pgd* locus presented three alleles *6-Pgd*$_{92}$, *6-Pgd*$_{100}$ and *6-Pgd*$_{108}$; in the population from PUR, the first two were observed; and in SIT, the last two, being the *6-Pgd*$_{100}$ allele the most frequent (Table 2). In TIBÚ, this locus was monomorphic for the *6-Pgd*$_{100}$ allele. Heterozygous individuals showed three bands, suggesting a dimeric protein structure. These results agree with a great part of the studies performed in *Anopheles* (Steiner *et al*., 1981; Narang *et al*., 1993; Fritz *et al*., 1995; Santos *et*

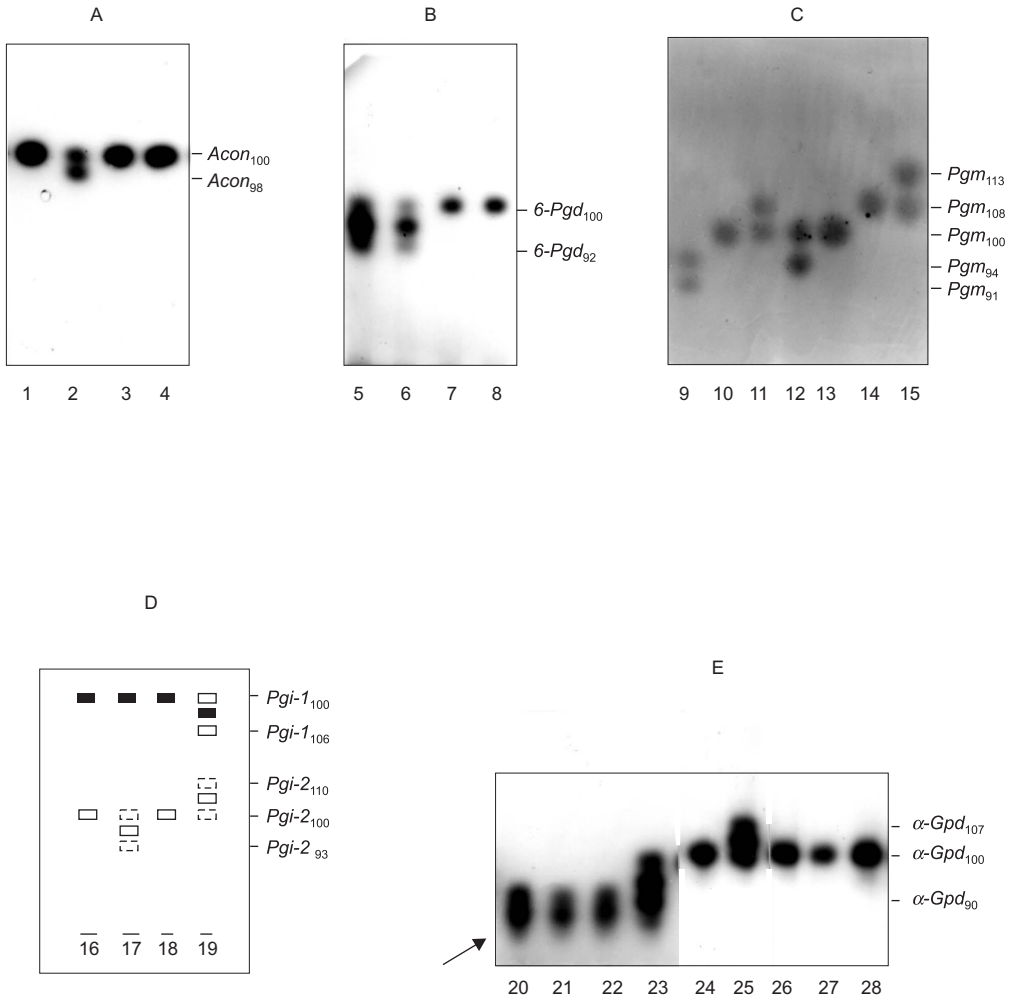

Fig. 2 — *Anopheles nuneztovari*. A) Aconitase. Samples from TUC. Phenotypes of the *Acon* locus: $Acon_{100}$ (1, 3, 4), $Acon_{100/98}$ (2). **B)** 6-Phosphogluconate dehydrogenase. Samples from TUC. Phenotypes of the *6-Pgd* locus: $6\text{-}Pgd_{100/92}$ (5, 6), $6\text{-}Pgd_{100}$ (7, 8). **C)** Phosphoglucomutase. Samples from BR. Phenotypes of the *Pgm* locus: $Pgm_{94/91}$ (9), Pgm_{100} (10, 13), $Pgm_{108/100}$ (11), $Pgm_{100/94}$ (12), Pgm_{108} (14), $Pgm_{113/108}$ (15). **D)** Phosphoglucose isomerase. Samples from TUC. Phenotypes of the *Pgi-1* locus: $Pgi\text{-}1_{100}$ (16 to 18), $Pgi\text{-}1_{100/96}$ (19), and phenotypes of the *Pgi-2* locus: $Pgi\text{-}2_{100}$ (16, 18), $Pgi\text{-}2_{100/93}$ (17), $Pgi\text{-}2_{110/100}$ (19). **E)** α-Glycerophosphate dehydrogenase. Samples from SIT (20 to 23) and TUC (24 to 28). Phenotypes of the α-*Gpd* locus: $\alpha\text{-}Gpd_{90}$ (20 to 22), $\alpha\text{-}Gpd_{100/90}$ (23), $\alpha\text{-}Gpd_{100}$ (24, 26 to 28), $\alpha\text{-}Gpd_{107/100}$ (25). The arrow points to the additional band.

al., 1996; Maia, 1997; among others). The exception is the *A. quadrimaculatus* complex, in which three loci were revealed with genetic variation in all of them (Narang *et al*., 1989).

Phosphoglucomutase

The phosphoglucomutase presented only one region (Fig. 2C). Genetic control for this zone was interpreted as depending on one locus *Pgm* with six codominant alleles: Pgm_{113}, Pgm_{108}, Pgm_{100}, Pgm_{94}, Pgm_{91} and Pgm_{89} (Table 2). The population from NOMA presented six alleles, while in three other populations from the Brazilian Amazon only the first five were detected. In the populations from TIBÚ and SIT only the Pgm_{108}

and Pgm_{100} alleles were verified. Heterozygous individuals showed two bands that indicate monomeric protein structure. The PGM enzyme has been extensively investigated in different insect groups, including the genus *Anopheles*. The results of this study are similar to those studies performed in other anopheline species, which described elevated genetic variation, and usually more than three alleles (Narang *et al*., 1989; 1993; Manguin *et al*., 1995).

Phosphoglucose Isomerase

Phosphoglucose isomerase presented two activity regions (Fig. 2D). It is probable that these zones are coded by two loci *Pgi-1* and *Pgi-2*. Genetic variation was exclusive to the TUC population, with two alleles in the first locus and three in the second (Table 2). Heterozygous individuals presented a profile with three bands, allowing to propose a dimeric structure for the protein.

Distinct results were obtained for *A. nuneztovari* from Suriname and Venezuela. Steiner *et al*. (1980) described a monomorphic locus in adults possibly due to differences in the genic expression between larva and adult stage. One locus was also found in *A. albitarsis* (Maia, 1997), *A. aquasalis* (Steiner *et al*., 1981), *A. quadrimaculatus* complex (Narang & Seawright, 1988), *A. punctulatus* complex (Foley *et al*., 1995), *A. deaneorum* and *A. marajoara* (Narang *et al*., 1993), and *A. pseudopunctipennis* (Manguin *et al*., 1995).

α-Glycerophosphate Dehydrogenase

Analyzed in adults, the α-GPD presented four electrophoretic profiles, interpreted as resulting from the presence of three alleles in the α-*Gpd* locus: Gpd_{107}, Gpd_{100} and Gpd_{90} (Fig. 2E; Table 2). The TUC population presented α-$Gpd_{107/100}$ and α-Gpd_{100} phenotypes; the BR, PUR and NOMA populations, α-Gpd_{100} phenotype; the TIBÚ population, α-Gpd_{100} and α-$Gpd_{100/90}$ phenotypes; and the population from SIT α-Gpd_{100}, α-$Gpd_{100/90}$ and α-Gpd_{90} phenotypes. Heterozygous individuals showed three bands, suggesting a dimeric protein structure. The α-Gpd_{90} phenotype, exclusive to the SIT population, presented one additional band, with slower migration in relation to the product of the α-Gpd_{90} allele. We suggest that its presence would have originated by post synthesis changes, implicating structural modifications in the molecule that codes the allele α-$Gpd_{90,}$ as it was absent in the unique homozygote individual found for the allele α-Gpd_{100} (Scarpassa *et al*., 1996). Interesting, this additional band also was present in larvae, pupae and adults (Scarpassa *et al*., 1996). Interesting, this ontogenetic pattern differs from the Brazilian Amazon populations (Scarpassa & Tadei, 1993).

Allelic variation in the α-*Gpd* locus was also noted in populations of *A. nuneztovari* from Suriname (Steiner *et al*., 1980) and from Tucuruí (Scarpassa & Tadei, 1993). However, in spite of the presence of genetic variation in populations of *A. nuneztovari*, one monomorphic locus has been observed in most *Anopheles* species (Narang *et al*., 1979; Lanzaro *et al*., 1995; Foley *et al*., 1995; among others).

The importance of the α-GPD in the flight of the insects led to the investigation of this enzyme in various species, and the results showed that α-GPD varies little (Johnson, 1974). The low frequency of heterozygous individuals observed in the α-*Gpd* locus, indicates that most mutants are harmful (reviewed in Scarpassa & Tadei, 1993). These conclusions are based on the premise that in insects this enzyme exerts an important metabolic role, producing energy for the maintenance of flight (O'Brien & MacIntyre, 1972).

In summary, in this study a total of 21 loci was detected, 16 of them were analyzed due their better resolution and accuracy with the electrophoretic conditions employed. Of the 16 loci, five (*Lap-2*, *Lap-4*, *Idh-2*, *Me* and *Xdh*) were monomorphic in the six populations studied. The highest variability was found in the *Pgm*, *Est-5* and *Acon* loci.

The data of this study are an important register of the electrophoretic profiles, including migration pattern in the gel, number of activity zones coded by different loci and their alleles of each enzyme studied in *A. nuneztovari*.

This makes it possible to compare them more accurately with patterns of other populations of *A. nuneztovari*, as well as of other anopheline species.

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