Variability and genetic differentiation among *Anopheles (Ano.) intermedius* Chagas, 1908 and *Anopheles (Ano.) mattogrossensis* Lutz & Neiva, 1911 (Diptera: Culicidae) from the Brazilian Amazon

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Anopheles (Anopheles) intermedius *and* Anopheles (Ano.) mattogrossensis *are Brazilian anopheline species belonging to the scarcely studied* Anopheles *subgenus. Few studies have been done on the genetic differentiation of these species. Both species have been found infected by* Plasmodium *and are sympatric with other anopheline species from the* Nyssorhynchus *subgenus. Eighteen enzymatic loci were analyzed in larval specimens of* An. intermedius *and* An. mattogrossensis *aiming to estimate the variability and genetic differentiation between these species.* An. mattogrossensis *population showed higher genetic variability (P = 44.4 and Ho = 0.081 ± 0.031) than that of* An. intermedius *(P = 33.3 and Ho = 0.048 ± 0.021). Most analyzed loci showed genotypic frequencies according to Hardy-Weinberg equilibrium, except for* LAP1 *and* LAP2 *in* An. intermedius*, and* EST1 *and* PGM *loci in* An. mattogrossensis*. The genetic distance between these species (D = 0.683) was consistent with the inter-specific values reported for* Anopheles *subgenus. We verified that the polymorphism and heterozygosity percentile values found in both species and compared to those in the literature, showed no relation between the level of isozyme variability and geographical distribution. The low variability found in these two species is probably more related to the niche they occupy than to their geographic distribution.*

Key words: *Anopheles intermedius* - *Anopheles mattogrossensis* - isozymes - malaria - Amazon

Information concerning variability and genetic differentiation in species of the subgenus *Anopheles* is very scanty in Brazil, even thought they include malaria vector species in other American neighboring countries (Rodriguez et al. 2000, Santos et al. 2003). *An. intermedius* and *An. mattogrossensis* show a wide geographical distribution in South America (Bolivia, Brazil, Peru, Colombia, Venezuela, Trinidad, and Guiana), being most abundant in forested areas like the Brazilian Amazon Region (Forattini 1962). Larvae are mostly found in shaded lagoons, marshes, floodplains, residual puddles in the forest, and river stretches in the middle of the bushes. Females are exophilic and zoophilic, and there have been no evidence of being able to transmit malaria (Forattini 1962, Belkin et al. 1971). However, dissection and ELISA analyses revealed that these species were infected by *Plasmodium* (Arruda et al. 1986, Tadei & Dutary-Thatcher 2000). In an area where anthropic modified environments are rapidly spreading like the Brazilian Amazon, this species maybe under vigilance as able to transmit malaria.

This work aims to analyze the isoenzimatic genetic variability of *An*. (*Ano*.) *intermedius* and *An*. (*Ano*.) *mattogrossensis* and to compare to results found for species of the subgenus *Nyssorhynchus*.

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MATERIALS AND METHODS

Specimens and collection sites - Wild-caught *An. mattogrossensis* adult females were collected on cattle-bait in Lake Janauari (right margin of the Negro River, near Manaus), Amazonas (03º08'00.5"S, 60º00'34.7"W). *An. intermedius* adult females were captured on human-bait in forest border areas close to domiciles (30 m approximately) of Highway Pacoval, km 4, Macapá, Amapá (00º02'19.8"N, 51º03'40.7"W), between 18:00 and 22:00 h. Females mosquitoes were isolated for individual oviposition. After hatching, larvae from individual progenies were maintained at 26º ± 1ºC until 4th instar when they were frozen at –70ºC and underwent isoenzymatic analyses, according to Santos et al. (1981). Fourth instar larvae were employed in all the systems, except for α-GPDH, in which adults were used. Morphologic identifications were done on egg and adults based on Cova-Garcia and Sutil (1977) and Consoli and Lourenço-de-Oliveira (1994).

Isoenzymatic analyses - Eighteen loci were analyzed: esterase (EST1, EST3 - E.C.3.1.1.1), leucine aminopeptidase (LAP1, LAP2, LAP3 - E.C.3.4.11.1), α-glycerophosphate dehydrogenase (α-GPD - E.C.1.1.1.8), phosphoglucose isomerase (PGI - E.C.5.3.1.9), alcohol dehydrogenase (ADH - E.C.1.1.1.1), aldehyde oxidase (AO - E.C.1.2.3.1), isocitrate dehydrogenase (IDH - E.C.1.1.1.42), 6-phosphogluconate dehydrogenase (6-PGD - E.C.1.1.1.44), malate dehydrogenase (MDH - E.C.1.1.1.37), malic enzyme (ME - E.C.1.1.1.40), hexokinase (HK1, HK2, HK3, HK4 - E.C.2.7.1.1) and phosphoglucomutase (PGM - E.C.5.4.2.2). Electrophoresis was performed in starch gel (12.5%) and starch-agarose (2 and 1%, respectively)

following Steiner and Joslyn (1979), Lima and Contel (1990) and Santos et al. (1996). For each locus, the most frequent electromorph was designated the 100 allele and all other alleles were measured relative to it.

Statistic analyses - Approximately three individuals from each of 30 progenies were used for each enzymatic system, in a total of 100 individuals. Genetic variability for each species was estimated using the mean number of alleles per locus, proportion of polymorphic loci (P), mean observed heterozygosity (Ho), and expected heterozygosity (He) using the Biosys-1 program (Swofford & Selander 1981). Conformance tests for Hardy-Weinberg equilibrium was done by Chi-square test using the Biosys-1 program and by G-square test (Monjeló 2005). The no-criteria option from the Biosys-1 program was used to estimate the percentage of polymorphic loci. Thus, a locus was considered polymorphic if any variation was observed, independent of the frequency of alleles detected. T-student test was used to assess the significance level of the mean observed and expected heterozygosities between *An. intermedius* and *An. mattogrossensis*. Genetic distance was calculated according to Nei (1978).

RESULTS

Five out of the 18 loci analyzed presented polymorphism for both species: EST1, LAP1, LAP2, LAP3, and PGM. PGM *locus* was the one presenting the largest number of alleles when considering both species (Table I). EST3, 6PGD, and MDH *loci* were polymorphic only for *An. mattogrossensis*, whereas IDH *locus* was polymorphic only for *An. intermedius*. IDH presented three alleles – IDH_{106}, IDH_{100} and IDH_{92}. In *An. mattogrossensis* only IDH_{92} was detected (Fig. 1). IDH_{92} was considered a diagnostic *locus*, as well as HK1, HK2, HK3, and HK4 *loci*, which were monomorphic for the $HK1_{100}$, $HK2_{100}$, $HK3_{100}$ and $HK4_{100}$ alleles in *An. intermedius* and for the $HK1_{94}$, $HK2_{95}$, $HK3_{94}$ and $HK4_{98}$ alleles in *An. mattogrossensis* (Fig. 2). For the PGM *locus*, the PGM_{100} allele was common in the two species (0.929 for *An. intermedius*). The PGM_{105} allele was only detected in *An. intermedius* while PGM_{97} was detected only in *An. mattogrossensis* (Table I).

Both species showed most *loci* in Hardy-Weinberg equilibrium as demonstrated by Chi-square and G-square results (Table I). For most *loci* χ^2 and G^2 were not significant except for *loci* LAP1 ($\chi^2 = 56.998$, $G^2 = 7.953$, D.F. = 1, $P < 0.01$) and LAP2 ($\chi^2 = 11.692$, $G^2 = 6.961$, D.F. = 1, $P < 0.01$) for *An. intermedius* population, and EST1 ($\chi^2 = 10.120$, $G^2 = 7.732$, D.F. = 1, $P < 0.01$) and PGM ($\chi^2 = 5.071$, $G^2 = 5.756$, D.F. = 1, $P < 0.05$) *loci* for *An. mattogrossensis* (Table I). Most of the Hardy-Weinberg equilibrium deviations were probably due to excess of observed homozygous individuals, in comparison with the expected numbers.

TABLE I

Isoenzymatic allelic frequency for 18 loci for *Anopheles intermedius* and *An. mattogrossensis*

Locus	Allele	Species	
		An. intermedius	*An. mattogrossensis*
EST1	n	101	106
	100	0.856	0.858
	98	0.144	0.142
$G^2_{H\text{-}W}$		1.461 ^{ns}	7.732 ^{a}
$\chi^2_{H\text{-}W}$		2.729 ^{ns}	10.120 ^{a}
EST3	n	101	93
	100	1.000	0.871
	98	0.000	0.129
$G^2_{H\text{-}W}$		-	0.210 ^{ns}
$\chi^2_{H\text{-}W}$		-	0.226 ^{ns}
LAP1	n	87	133
	100	0.983	0.970
	97	0.017	0.030
$G^2_{H\text{-}W}$		7.953 ^{a}	1.658 ^{ns}
$\chi^2_{H\text{-}W}$		56.998 ^{a}	0.111 ^{ns}
LAP2	n	90	131
	100	0.917	0.870
	97	0.083	0.130
$G^2_{H\text{-}W}$		6.961^{a}	1.786 ^{ns}
$\chi^2_{H\text{-}W}$		11.692 ^{a}	2.089 ^{ns}

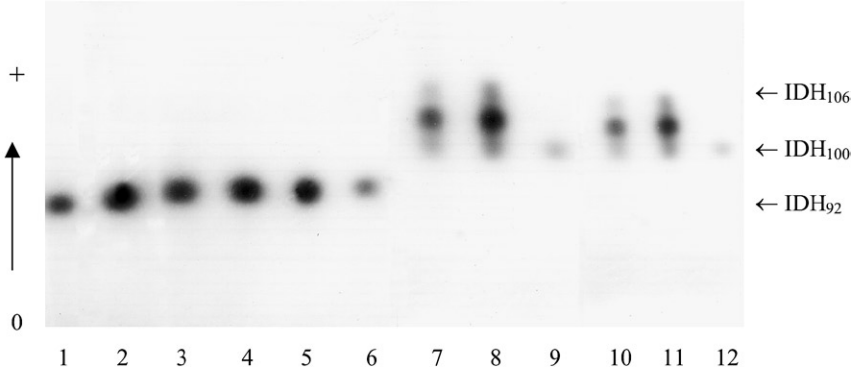

Fig. 1: starch gel electrophoresis isocitrate dehydrogenase isoenzymatic profiles for 4th-instar larvae of *Anopheles mattogrossensis* (samples 1 to 6) and *An. intermedius* (samples 7 to 12). Tris-citrate buffer system, pH 7.1.

Locus	Allele	Species	
		An. intermedius	*An. mattogrossensis*
LAP3	n	77	114
	100	0.870	0.952
	96	0.130	0.048
$G^2_{H\text{-}W}$		0.065 *ns*	0.688 *ns*
$\chi^2_{H\text{-}W}$		0.061 *ns*	0.265 *ns*
IDH	n	123	100
	106	0.016	0.000
	100	0.956	0.000
	92	0.028	1.000
$G^2_{H\text{-}W}$		1.012 *ns*	-
$\chi^2_{H\text{-}W}$		0.244 *ns*	-
6PGD	n	117	101
	106	0.000	0.119
	100	1.000	0.881
$G^2_{H\text{-}W}$		-	0.331 *ns*
$\chi^2_{H\text{-}W}$		-	0.365 *ns*
MDH	n	104	113
	100	1.000	0.987
	94	0.000	0.013
$G^2_{H\text{-}W}$		-	0.028 *ns*
$\chi^2_{H\text{-}W}$		-	0.014 *ns*
PGM	n	106	103
	105	0.071	0.000
	100	0.929	0.728
	97	0.000	0.272
$G^2_{H\text{-}W}$		1.066 *ns*	5.756 *b*
$\chi^2_{H\text{-}W}$		0.571 *ns*	5.071 *b*
HK1	n	104	101
	100	1.000	0.000
	94	0.000	1.000
HK2	n	104	101
	100	1.000	0.000
	95	0.000	1.000
HK3	n	104	101
	100	1.000	0.000
	94	0.000	1.000
HK4	n	104	101
	100	1.000	0.000
	98	0.000	1.000

ME, PGI, ADH, AO, and α-GPD were monomorphic for the two species. Degrees of freedom is equal to one for all *loci* except IDH *locus* (DF = 3). n: sample size; *ns*: not significant; *a*: $P < 0.01$; *b*: $P < 0.05$; -: not calculated.

Two-banded heterozygotes were stained in EST, LAP, and PGM indicating a monomeric structure for these enzymes, and IDH, 6PGD, and MDH showed three bands in the heterozygotes, a pattern typical of dimeric enzymes. The enzymes ME, HK, PGI, ADH, AO, and α-GPD were monomorphic for both species (i.e. no heterozygote individual was detected).

The results of the average number of samples and of alleles per *locus* were similar between *An. intermedius* and *An. mattogrossensis* species (95.7 ± 4.8 and 98.4 ± 5.5; 1.4 ± 0.1 and 1.4 ± 0.1, respectively). Higher polymorphism and heterozygosity were found in *An. mattogrossensis* (P = 44.4; He = 0.081 ± 0.031). *An. intermedius* presented lower polymorphism (P = 33.3) and heterozygosity (He = 0.048 ± 0.021) values (Table II). However, there is not a statistically significant difference between the observed and expected mean heterozygosities of the two species (Student's t = –0.876, –0.956, and P = 0.389, 0.348, respectively).

A comparative analysis of genetic variability parameters between species of the subgenera *Nyssorhynchus* and *Anopheles*, based on the present work and published data, is shown in Table III. Genetic distance index between *An. intermedius* and *An. mattogrossensis* was 0.683, in conformity with previous values found for interspecific variation in the genus *Anopheles*.

DISCUSSION

An. intermedius and *An. mattogrossensis* are species belonging to the subgenus *Anopheles*. Because these species do not present epidemiological importance and are of restrict geographical distribution in Brazil, little is known about their population genetics. Isoenzymatic analyses found about 30% polymorphic *loci* for both species out of 18 *loci*. Five monomorphic *loci* were considered diagnostic, i.e. could separate *An. intermedius* from *An. mattogrossensis*. These were IDH1, HK1, HK2, HK3, and HK4. Fritz et al. (1995) identified three diagnostic *loci* for AO, IDH2, and ME, which separate *An. nuneztovari, An. rangeli,* and *An. trinkae*, species of the *Nys-sorhynchus* subgenus, with a closer phylogenetic relationship between the latter two. IDH1 and IDH2 also separated the A and B forms of *An. quadrimaculatus* complex (Lanzaro et al. 1990). According to these authors, the analyses of the genotypic frequencies in these two *loci* revealed a significant deficiency of heterozygotes, consistent with the Wahlund effect, observed when sympatric populations with limited

TABLE II

Genetic variability estimate at 18 *loci* of the *Anopheles intermedius* and *An. mattogrossensis* populations

Population	Mean sample size/locus	Mean no. of alleles/*locus*	% polymorphic *loci* [a]	Mean heterozygosity	
				Observed	Expected [b]
An. intermedius	95.7 ± 4.8	1.4 ± 0.1	33.3	0.048 ± 0.021	0.049 ± 0.020
An. mattogrossensis	98.4 ± 5.5	1.4 ± 0.1	44.4	0.081 ± 0.031	0.082 ± 0.029
Student's t				–0.876 (26) P = 0.389 *ns*	–0.956 (26) P = 0.348 *ns*

a: a locus was considered polymorphic if more than one allele was detected (Swofford & Selander 1981); *b*: expected heterozygosity of Hardy-Weinberg; Nei's unbiased estimate (Nei 1978); *ns*: not significant; degrees of freedom within parentheses.

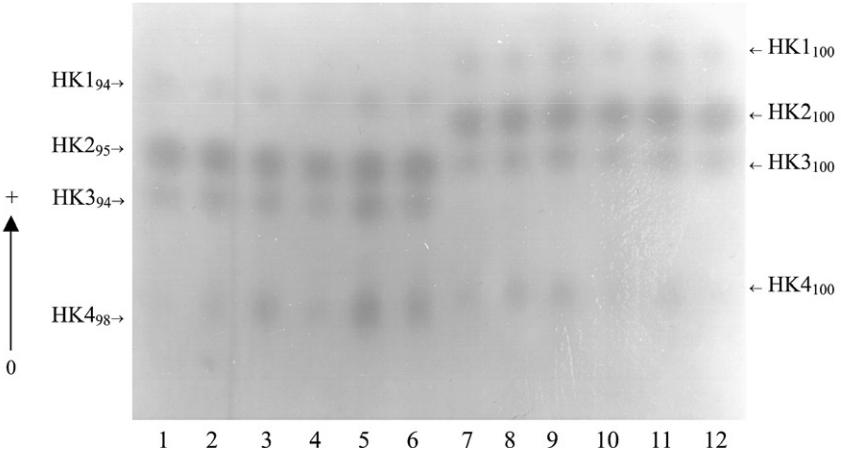

Fig. 2: starch gel electrophoresis of hexokinase isoenzymatic profiles for 4th-instar larvae of *Anopheles mattogrossensis* (samples 1 to 6) and *Anopheles intermedius* (samples 7 to 12). Tris-citrate buffer system, pH 7.1.

gene flow are considered as a unique population. However, the lack of heterozygotes found in three of the four *loci* for *An. intermedius* and *An. mattogrossensis* cannot be accounted for the Wahlund effect, since populations come from different sites apart 2000 km approximately.

Polymorphism values observed for *An. intermedius* (P = 33.3) and *An. mattogrossensis* (P = 44.4) are similar to those found for other species of the subgenus *Anopheles*, such as *An. franciscanus* (P = 33.3) and *An. crucians* (P = 39.4) (Manguin et al. 1995). However, in the subgenus *Nyssorhynchus*, high levels of polymorphism (P > 55.0) were reported for one Brazilian population of *An. albitarsis* (Narang 1980), three populations of *An. albimanus* from the Northern Colombia (Narang et al. 1991) and four populations of *An. darlingi* from the Brazilian Amazon (Santos et al. 1999). Carvalho-Pinto and Lourenço-de-Oliveira (2004) found higher polymorphism levels on *An. cruzii* Brazilian populations of the subgenus *Kerteszia* (P = 72.7 - 81.8). Extensive genetic variability in mosquitoes is thought to have implications on malaria control programs, since it could represent plasticity to respond to selection pressure of insecticides (Tadei 1993). Small polymorphism values were registered for Southern Colombian populations of *An. albimanus* ($16 \leq P \leq 40$). *An. albimanus* low polymorphism values could be due to the relation between the habitat and the reduced population size in the estuaries, which can promote inbreeding, resulting in genetic drift or marginal effect and selection. Low polymorphism values were also detected in the *An. triannulatus* population from Lake Janauari ($25 \leq P \leq 56.3$, Brazilian Amazon, Santos et al. 2004), in *An. nuneztovari* from Highway BR-174-AM, km 204 (P = 31.3, Brazilian Amazon, Scarpassa et al. 1999), and *An. aquasalis* population from Rio de Janeiro (P = 34, Narang 1980), of restricted distribution in brackish water on estuarine habitats. For the *An. aquasalis* population, polymorphism value was similar to that found for *An. intermedius* (Table III). The low polymorphism in *An. triannulatus* and *An. nuneztovari* populations (Scarpassa et al. 1999, Santos et al. 2004) could be related to the fact that collection was performed during dry season, when breeding sites are drastically reduced, leading to mosquitoes population density decrease and favoring inbreeding, that could result in genetic drift or marginal effect and, selection. Low genetic variation was also found in *An. mattogrossensis* based on mitochondrial DNA-sequencing, when compared with six anopheline species of the subgenus *Nyssorhynchus*. The same study also showed lower adenine and thymine rates on *An. mattogrossensis* specimens than on those from subgenus *Nyssorhynchus*, possibly suggesting a lower mutation rate (Borges R and Santos JMM, unpublished data).

Of the six polymorphic *loci* found in *An. intermedius*, only LAP1 and LAP2 were significant (Table I). Deviation from Hardy-Weinberg equilibrium was due in part to the large number of homozygotes found. In *An. mattogrossensis*, two *loci* (EST 1 and PGM) also presented significant deviations from their expected allelic frequencies. For EST1 was due to heterozygote deficiency. For PGM, deviation was related to heterozygote excess. Heterozygote deficiency was seen in an *An. albitarsis* population from Macapá (Amapá) for EST4 and LAP1 *loci* (Maia 1997). When studying *An. nuneztovari* populations from Brazil and from Colombia, Hardy-Weinberg disequilibrium was found for some *loci*, favoring homozygotes excess for a rare allele of EST5 and PGM *loci*, or heterozygotes individuals constituted by two rare alleles of IDH1 and MDH *loci* (Scarpassa et al. 1999). A large number of homozygotes was verified for some *loci* in *An. darlingi* populations from the Amazon Region, except for the EST2 *locus* in the Manaus population, in which the heterozygotes were more frequent (Santos et al. 1999).

Lower heterozygosity values were obtained for *An. intermedius* and *An. mattogrossensis* when compared to species belonging to *Nyssorhynchus* and *Anopheles* subgenera (Table III). However, difference in the mean heterozygosity values for *An. intermedius* and *An. mattogrossensis* is not great enough to reject the possibility that the difference is due to random sampling variability.

TABLE III

Comparison of the genetic variability values (mean number of alleles per *locus*, polymorphic *loci* and heterozygosity) for *Anopheles* species of *Nyssorhynchus*[1] and *Anopheles*[2] subgenus

Species		Mean no. of alleles/*locus*	% polymorphic *loci* [a]	Mean heterozygosity		References
				Observed	Expected [c]	
An. darlingi [1]		1.89 ± 0.22 - 2.26 ± 0.27	52.63 - 63.15[a]	0.236 ± 0.09 - 0.432 ± 0.11	0.290 ± 0.11 - 0.375 ± 0.08	Santos et al. (1999)
		-	63.2[b]	0.125	-.	Narang (1980)
An. albitarsis [1]		1.5 ± 0.2	40.9[a]	0.085 ± 0.034	0.099 ± 0.038	Maia (1997)
		-	65.0[b]	0.170	-	Narang (1980)
An. nuneztovari [1]		1.8 ± 0.3 – 2.1 ± 0.3	31.3 - 56.3[a]	0.078 ± 0.04 - 0.117 ± 0.06	0.087 ± 0.06 - 0.116 ± 0.06	Scarpassa et al. (1999)
		-	54.0[b]	0.111	-	Narang (1980)
An. albimanus [1]		1.3 ± 0.1 - 3.6 ± 0.4	16.0 – 60.0[b]	0.05 ± 0.03 - 0.22 ± 0.04	-	Narang et al. (1991)
An. argyritarsis [1]		-	68.2[b]	0.113	-	Narang (1980)
An. evansae [1]		-	63.0[b]	0.149	-	Narang (1980)
An. aquasalis [1]		-	34.0[b]	0.084	-	Narang (1980)
An. triannulatus [1]		1.4 ± 0.2 - 1.9 ± 0.2	25.0 - 56.3[a]	0.077 ± 0.05 - 0.133 ± 0.05	0.076 ± 0.05 - 0.174 ± 0.06	Santos et al. (2004)
An. pseudopunctipennis[2]		1.1 ± 0.1 - 2.5 ± 0.2	12.1 - 78.8[b]	0.003 ± 0.002 - 0.101 ± 0.03	0.003 ± 0.002 - 0.10 ± 0.03	Manguin et al. (1995)
An. franciscanus[2]		1.4 ± 0.1	33.3[b]	0.084 ± 0.027	0.088 ± 0.028	Manguin et al. (1995)
An. crucians[2]		1.5 ± 0.2	39.4[b]	0.078 ± 0.029	0.071 ± 0.025	Manguin et al. (1995)
		2.38 - 2.62	58.5 - 58.8[b]	0.163 ± 0.03 - 0.179 ± 0.05	-	Steiner et al. (1975)
An. quadrimaculatus[2]	**A**	3.43	73.0[b]	0.230	-	
	B	2.73	50.0[b]	0.180	-	
	C	2.80	60.0[b]	0.210	-	Narang et al. (1989)
An. intermedius[2]		1.4 ± 0.1	33.3[a]	0.048 ± 0.021	0.049 ± 0.020	
An. mattogrossensis[2]		1.4 ± 0.1	44.4[a]	0.081 ± 0.031	0.082 ± 0.029	

a: a locus was considered polymorphic if more than allele was detected; *b*: a locus was considered polymorphic if the frequency of the most common allele did not exceed 0.99 (Morton et al. 1966); *c* : expected heterozygosity of Hardy-Weinberg; Nei's unbiased estimate (Nei 1978).

Species or populations distributed in a large variety of environmental conditions would probably be more genetically heterozygous and/or polymorphic. Contrarily, populations of limited or restricted distribution in special habitats would be less polymorphic (Narang 1980). Low heterozygosity (0.084) found in *An. aquasalis* suggested this to be an strategy of the species, because larvae breed in brackish water, mainly restricted to the coastal region (Narang 1980).

High heterozygosity values were found for *An. crucians* (Steiner et al. 1975) and species A, B, and C of the *An. quadrimaculatus* complex (Narang et al. 1989), that are vectors species of the subgenus *Anopheles*. However, the *An. pseudopunctipennis* population from Granada Island differed from those in the continent on account of lacking heterozygotes and low polymorphism, due to their geographical isolation, suggesting this low level of genetic variability to be brought about by "founder effect" (Manguin et al. 1995).

Data on genetic distance values (D = 0.683) of *An. intermedius* and *An. mattogrossensis* are consistent with those found in the literature for interespecific variation in *Anopheles* subgenus (Manguin et al. 1995). These authors identified lower distance values (D = 0.335) between the closely related species, *An. pseudopunctipenis* and *An. franciscanus*, when compared with *An. crucians* (D = 0.997). Higher value (D = 2.355) was observed between *An. pseudopunctipenis* and *An.* (*Nys*.) *albimanus*. However, Santos et al. (2003) found lower values (D = 0.373 - 0.989) among five *Anopheles* species belonging to the *Nyssorhynchus* and *Anopheles* subgenera from the Amazon Region. In *An. rangeli* and *An. trinkae*, the genetic distance ranged from $0.149 \leq D \leq 0.197$ and between these two species and *An. nuneztovari* it ranged from $0.286 \leq D \leq 0.440$ (Fritz et al. 1995), both of the *Nyssorhynchus* subgenus.

The genetic identity (I = 0.382) found between *An. intermedius* and *An. mattogrossensis* agrees with the values proposed by Avise (1974) for cogeneric populations ($0.26 \leq I \leq 0.67$).

Low heterozygosity and isoenzyme polymorphism found in *An. intermedius* and *An. mattogrossensis* populations agree with data found in literature for species of restricted distribution of the subgenus *Anopheles* and some of the subgenus *Nyssorhynchus*, and it differs from most malaria vector species. Nonetheless, *An. intermedius* and *An. mattogrossensis* can be considered highly specialized species since they can be found only in restricted spotted niches in spite of showing a wide geographical distribution. *An. intermedius* is found mainly in the border between the primary forest and cattle pastures, while *An. mattogrossensis* is found in swamps or shallow flooded areas. The low variability found in these two species is probably related to the specialized ability to thrive on the niches they occupy rather than to their large geographical distribution. The low variability can also be a result of *An. intermedius* and *An. mattogrossensis*' genetic structures, where selection pressures were not enough to lead to higher variability.

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