GENETIC STRUCTURE OF NATURAL POPULATIONS OF *ANOPHELES ALBIMANUS* IN COLOMBIA

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ABSTRACT. Electrophoretic and cytogenetic studies were undertaken on the population structure of *Anopheles albimanus* from 11 localities in Colombia, 3 from northern (Atlantic coast) and 8 from southern (Pacific coast) regions. Of the 25 allozyme loci examined, significant allele frequency differences were observed at 4 loci: hydroxy acid dehydrogenase (Had-1) and 3 esterases (Est-2, Est-4 and Est-6). The northern populations had higher variability, with 55% polymorphic loci, a mean heterozygosity of 20.4% and a mean of 3.0 alleles per locus. These values for southern populations were 24%, 9.1% and 1.5 %, respectively. There were neither diagnostic loci nor clinal effect on frequencies of allozymes. Except for a small inversion on the X chromosome in low frequency in certain populations, all populations were homosequential in chromosomal banding patterns. Hybrids from matings between natural populations and the Gainesville laboratory strain were fully fertile. Estimates of genetic similarities (0.95–0.97 among southern and 0.99–1.00 among northern populations) suggest a lack of significant genetic differentiation among distant populations in this species. Based on the chromosomal, hybridization and electrophoretic data, we concluded that mosquitoes from the 11 collections were conspecific populations of *An. albimanus*.

INTRODUCTION

Anopheles albimanus Wied., a neotropical species in the subgenus *Nyssorhynchus*, has been incriminated as the most important vector of human malaria over most of Central and part of South America. In Colombia, this species is widely distributed throughout the northern part of the country extending up to several hundred kilometers into the interior in the Magdalena River Valley, west of the Andes Mountains, and along the Pacific coast south to Peru. Ecological conditions throughout this range vary greatly in relative humidity, precipitation (varying from the world's highest rainfall in some Pacific coast areas to arid and semi-arid areas in the north), vegetation and breeding site characteristics. Due to the widespread occurrence of resistance to pesticides among natural populations of *An. albimanus*, research efforts have shifted from development of chemical control techniques to integrated pest management strategies including genetic control.

We attempted to elucidate the population structure of *An. albimanus* and identify possible sympatric or allopatric cryptic species by electrophoretic and cytogenetic methods. To our knowledge, there is no published report on the type, pattern and extent of genetic variation in this species. In this paper, we describe allozyme, chromosomal and hybridization studies on 11 populations of *An. albimanus* collected from various sites along the Atlantic and Pacific coasts of Colombia.

MATERIALS AND METHODS

Natural populations (larvae and adults) of *An. albimanus* were collected from 11 localities in Colombia (Fig. 1). Two major cities, Cartagena (a port city in the northwest part of Colombia) and Tumaco (in the deep south along the Pacific Coast) are included on the map to serve as reference points for collection sites. In the north, the Arjona (AR) and Carmen De Bolivar (CB) populations were from freshwater breeding sites, and Monito (MON) was a saltwater (only larvae collected) population. In the south, Pizzaro (PIZ) and Orpua (ORP) were from the estuaries north of the port of Buenaventura. The Gamboa (GAM) population was collected from an abandoned shrimp breeding pond within the city limit of Buenaventura. The 5 localities south of Buenaventura included Punta Bonita (PB), Periquillo (PER), Puerto Merizalde (PM), Salahonda (SAL) and Ica (ICA). The last 2 sites are located in the region of Tumaco (Fig. 1). The larvae were reared to adults in the laboratory and were stored along with the adult collections at −70°C until used for electrophoresis.

Starch-gel electrophoresis was conducted according to Narang et al. (1989a). Three buffer systems were used to analyze 17 enzyme systems comprising 25 allozyme loci: 1) tris-citrate buffer, pH 7.0 (gel buffer: 0.009M tris, 0.003M citric acid, pH 7.0; electrode buffer: 0.135M tris, 0.045M citric acid, pH 7.0) [CA-7 of Steiner and Joslyn (1979)]: adenylate kinase (ADK) alde-

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Fig. 1. Collection sites of *Anopheles albimanus* along the Atlantic and Pacific costs of Colombia.

hyde oxidase (AO), esterases (EST), hydroxy acid dehydrogenase (HAD) and 6-phosphogluconate dehydrogenase (6PGD); 2) tris-citrate buffer, pH 5.5 (gel buffer: 0.02M tris, 8 mM citric acid, pH 5.5; electrode buffer: 0.16 M tris, 0.07 M citric acid, pH 5.2): aconitase (ACON) hexokinase (HK), a-glycerophosphate dehydrogenase (a-GPDH), malate dehydrogenase, (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI) and phosphoglucomutase (PGM); and 3) tris-citrate buffer, pH 7.6 (gel buffer: 0.002 M tris, 0.004 M citric acid, pH 7.6; electrode buffer: 0.22 M tris, 0.05 M citric acid, pH 7.6): peptidase (PEP), glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), mannose phosphate isomerase (MPI) and xanthine dehydrogenase (XDH).

A laboratory stock, BM-1 (isolated from a mass-reared colony which originated from 7 samples collected from El Salvador about 18 years ago), homozygous for 25 loci, was standard reference for identification of electromorphs in natural populations. This stock was homozygous for the electromorph (at each locus) which was most common in the colony. The electromorph of each locus of BM-1 was given a mobility value of 100, and the *Rf* values of the electromorphs of the corresponding locus in natural populations were calculated relative to BM-1.

Statistical analysis of electrophoretic variability data (number of each genotype at each locus) was performed by using the Fortran IV computer program BIOSYS 1 (Swofford and Selander 1981). The tests included conformance of observed electromorph frequencies to those expected under Hardy-Weinberg equilibrium (expected frequencies for small samples at some loci corrected by using Levene's (Levene 1949) formula); χ^2 test with pooling of genotypes to compensate for low expected frequencies of some classes; and linkage disequilibrium between loci by contingency χ^2 analysis and Nei's (1978) genetic identity and distance matrices for estimating phenetic relationships among natural populations.

Salivary gland chromosomes were prepared from live fourth-instar larvae soon after collection, according to the techniques of Kaiser et al. (1982) with a few modifications. In some cases, gravid females from various localities (CB, AR, GAM) were used to colonize strains (in the laboratory of SEM in Bogota, Colombia); and larvae of F_1 and F_2 generations were used for chromosomal analysis. In other cases, larvae were transferred to DMSO in Eppendorf tubes, transported to the USDA-ARS laboratory at Gainesville in dry ice and stored at −70°C until used for chromosome preparations. The glands were dissected in 45% acetic acid and stained in 2% lacto-acetic orcein (La Fevre 1976) diluted 1:2 with lactic acid (85%) to improve spreading of chromosomes and resolution of bands. The standard map of Keppler et al. (1973) was used as a reference for comparison of the banding patterns among natural populations.

Hybridization data reported in this paper were primarily collected from parental and backcrosses set up for linkage group analysis of mutants and allozymes. Hybridization experiments involved matings between laboratory reared (for 2–3 generations) field strains (from CB, AR, GAM and various localities near Tumaco) and an inbred laboratory strain (INS) maintained at the National Institute of Health, Bogota, originally colonized from wild mosquitoes from CB. The F_1 progeny were crossed to a multiple marker (allozymes and mutants) strain, BM-1 from Gainesville. Adults in most crosses were allowed to mate freely in cages, but in some cases an induced copulation method (Baker et al. 1962) was used for hybridization crosses.

RESULTS

Allozyme variability: Of the 25 loci, eight (*aGpdh-1, Acon-2, Adk* (3 loci), *Go-2, Got-1* and *Hk-2*) were monomorphic in all populations. Of the remaining 17 loci, three, *Pgi-1, Hk-1* and

Table 1. Frequencies of electromorphs of some polymorphic loci in 11 natural populations of *Anopheles albimanus*.

	Populations										
Locus	CB	MON	AR	GAM	ORP	PM	PIZ	ICA	PER	PB	SAL
Had-1											
(*n*)	122	51	24	25	40	44	30	40	28	96	36
121	0	0.02	0	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0	0	0	0	0
100	0.41	0.51	0.21	0	0	0.01	0.05	0.03	0.02	0.03	0
96	0.01	0	0	0	0	0	0	0	0	0	0
82	0.54	0.47	0.71	1.00	1.00	0.97	0.95	0.97	0.98	0.97	0.96
62	0.01	0	0	0	0	0	0	0	0	0	0.04
40	0.03	0	0.08	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0.02	0	0	0	0	0
Pgm-1											
(*n*)	185	83	32	51	57	40	39	31	39	194	35
146	0.02	0.04	0.02	0	0	0	0	0	0	0.01	0
134	0	0	0	0	0	0	0	0	0.01	0.01	0
125	0	0	0	0	0	0	0	0	0	0.01	0
120	0.85	0.86	0.79	1.00	1.00	1.00	0.99	1.00	0.99	0.97	1.00
105	0.01	0	0	0	0	0	0	0	0	0	0
100	0.07	0.08	0.11	0	0	0	0.01	0	0	0	0
94	0.01	0	0	0	0	0	0	0	0	0	0
86	0.02	0.02	0.06	0	0	0	0	0	0	0	0
79	0.01	0	0	0	0	0	0	0	0	0	0
76	0.01	0	0.02	0	0	0	0	0	0	0	0
Idh-1											
(*n*)	139	74	54	81	40	38	31	38	65	157	45
130	0.01	0	0	0	0	0	0	0	0	0	0
119	0.04	0	0	0	0.03	0.07	0.07	0	0	0.04	0
110	0.01	0	0	0	0	0	0	0	0	0	0
100	0.93	1.00	1.00	1.00	0.97	0.93	0.93	1.00	1.00	0.96	1.00
75	0.10	0	0	0	0	0	0	0	0	0	0
Mdh-1											
(*n*)	81	49	46	74	36	26	28	31	37	92	32
135	0.05	0.03	0	0	0	0.02	0	0	0	0	0
121	0.06	0.05	0.01	0	0	0	0	0	0.08	0.08	0.05
110	0	0.01	0	0	0	0	0	0	0	0	0
100	0.86	0.91	0.99	1.00	1.00	0.96	1.00	1.00	0.92	0.88	0.95
94	0.03	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0.02	0	0	0	0.04	0
Pep-4											
(*n*)	85	36	65	45	34	28	29	48	36	126	44
194	0.01	0.17	0.08	0.03	0	0	0	0.02	0	0.01	0
180	0.01	0.14	0.01	0	0	0	0	0	0	0	0
116	0	0	0	0	0	0	0	0.02	0	0.01	0.03
100	0.94	0.69	0.90	0.97	1.00	1.00	1.00	0.96	0.93	0.93	0.87
69	0.01	0	0.01	0	0	0	0	0	0.07	0.01	0
46	0.03	0	0	0	0	0	0	0	0	0.04	0.10
6-Pgd											
(*n*)	120	68	39	30	41	39	33	26	35	115	34
145	0.03	0.03	0	0	0	0	0	0	0	0	0
124	0.10	0.03	0.08	0	0	0	0	0	0	0.04	0
116	0.06	0.05	0.06	0	0.01	0.01	0	0	0	0.01	0
109	0.02	0	0	0	0	0	0	0	0	0.01	0
106	0.04	0	0	0	0	0	0	0	0	0.03	0
100	0.70	0.81	0.82	1.00	0.98	0.98	1.00	0.94	1.00	0.91	0.96
78	0.05	0.07	0.04	0	0.01	0.01	0	0.06	0	0	0.04
32	0	0.01	0	0	0	0	0	0	0	0	0

Table 1. continued

Locus	Populations										
	CB	MON	AR	GAM	ORP	PM	PIZ	ICA	PER	PB	SAL
Acon-1											
(*n*)	118	72	32	33	45	33	26	23	27	120	35
108	0.06	0	0.06	0	0	0	0	0	0	0	0
103	0.03	0.06	0	0	0	0	0	0	0.02	0.01	0
100	0.76	0.86	0.80	1.00	1.00	1.00	1.00	1.00	0.98	0.96	1.00
96	0.01	0	0	0	0	0	0	0	0	0.01	0
94	0.03	0	0.02	0	0	0	0	0	0	0.01	0
92	0.11	0.08	0.12	0	0	0	0	0	0	0.01	0
Ao-1											
(*n*)	101	67	38	84	36	27	22	28	34	95	20
103	0	0	0	0	0.01	0.02	0	0	0	0.01	0
100	0.03	0.07	0.05	0.01	0.10	0.04	0.14	0	0.06	0.03	0.05
95	0.95	0.91	0.86	0.98	0.89	0.94	0.86	1.00	0.91	0.95	0.92
85	0	0	0.04	0.01	0	0	0	0	0	0	0
48	0.02	0.02	0.05	0	0	0	0	0	0.03	0.01	0.03
Est-2											
(*n*)	41	58	45	40	26	32	24	25	27	40	27
104	0.01	0	0	0	0	0	0	0	0	0	0
103	0.04	0.19	0.09	0	0	0	0	0	0	0	0
100	0.72	0.52	0.60	0.95	1.00	0.69	1.00	1.00	1.00	0.90	1.00
97	0.21	0.29	0.30	0.05	0	0.19	0	0	0	0.10	0
95	0	0	0	0	0	0.12	0	0	0	0	0
88	0.02	0	0.01	0	0	0	0	0	0	0	0
Est-4											
(*n*)	55	38	24	40	36	32	28	25	28	60	25
116	0.02	0	0	0	0	0	0	0	0	0	0
106	0.08	0.03	0	0	0.06	0	0	0	0	0.23	0
103	0	0	0	0	0	0	0	0	0	0.02	0
100	0.17	0.39	0.15	0.78	0.75	1.00	0.80	1.00	0.95	0.67	0.90
95	0.73	0.58	0.81	0.22	0.19	0	0.18	0	0.05	0.08	0.10
89	0	0	0.04	0	0	0	0.02	0	0	0	0
Est-6											
(*n*)	58	49	39	32	32	30	25	23	23	25	28
119	0.02	0.01	0	0	0	0	0	0	0	0	0
113	0.02	0	0	0	0	0.20	0.18	0.30	0.33	0	0.11
107	0.34	0.27	0.33	0.63	0.41	0.18	0	0	0.04	0.70	0
100	0.35	0.35	0.39	0.31	0.59	0.62	0.82	0.61	0.63	0.28	0.89
94	0.27	0.37	0.28	0	0	0	0	0.09	0	0	0
90	0	0	0	0.06	0	0	0	0	0	0.02	0
Est-8											
(*n*)	65	73	35	36	34	51	27	28	30	38	28
162	0	0	0	0.08	0	0	0	0	0	0.08	0
153	0.06	0.10	0.06	0	0.16	0.18	0.17	0	0.03	0	0
142	0	0	0	0.12	0	0	0	0	0	0.15	0.25
137	0.19	0.09	0.19	0.24	0.27	0.32	0.28	0.29	0.37	0.24	0.29
116	0.50	0.44	0.54	0.28	0.57	0.44	0.55	0.71	0.33	0.29	0.36
100	0.25	0.37	0.21	0.28	0	0.06	0	0	0.27	0.24	0.10
Sodh											
(*n*)	28	27	30	39	41	37	35	31	36	48	33
120	0.05	0	0.03	0	0	0	0	0	0	0.04	0
100	0.87	0.98	0.91	0.99	1.00	1.00	1.00	1.00	1.00	0.64	0.88
79	0	0	0	0	0	0	0	0	0	0.26	0
72	0.04	0.02	0.03	0.01	0	0	0	0	0	0	0.08
52	0.04	0	0.03	0	0	0	0	0	0	0.06	0.04
Xdh-2											
(*n*)	48	29	28	29	26	47	24	26	29	66	31
117	0	0	0	0	0	0.23	0	0	0	0.01	0
109	0.03	0	0.11	0.02	0	0	0	0.04	0.04	0.03	0
100	0.96	0.98	0.82	0.86	1.00	0.77	0.98	0.96	0.79	0.88	0.89
88	0.01	0.02	0.07	0.12	0	0	0.02	0	0.17	0.08	0.11

Table 2. Estimates of genetic variability measurements in natural populations of *Anopheles albimanus* (standard error in parenthesis).

Population	Mean sample per locus	Mean no. alleles per locus	Percentage of loci polymorphic	Mean heterozygosity
Northern				
CB	89.1 (14.1)	3.6 (0.4)	56.0	0.22 (0.04)
AR	33.2 (1.9)	2.7 (0.3)	60.0	0.20 (0.04)
MON	48.7 (4.1)	2.6 (0.3)	48.0	0.20 (0.05)
Southern				
ORP	37.0 (1.4)	1.4 (0.2)	16.0	0.07 (0.03)
PIZ	27.6 (0.8)	1.4 (0.1)	24.0	0.07 (0.03)
GAM	40.8 (3.5)	1.6 (0.2)	20.0	0.10 (0.04)
PB	83.3 (9.3)	2.7 (0.3)	40.0	0.16 (0.04)
ICA	28.0 (1.0)	1.3 (0.1)	16.0	0.05 (0.03)
PM	34.2 (1.4)	1.9 (0.2)	28.0	0.12 (0.04)
PER	32.1 (1.7)	1.6 (0.2)	28.0	0.08 (0.04)
SAL	31.6 (1.3)	1.7 (0.2)	28.0	0.09 (0.03)

Me-1, revealed low level of polymorphism in one or more of the 3 northern populations, but these loci were monomorphic in southern populations. *Pgi-1* was polymorphic only in AR, with frequencies of 0.92 and 0.08 for allelomorphs with *Rfs* of 100 and 108, respectively. The frequencies of *Hk-1* alleles, *Rfs* of 138, 116, 109 and 92, were 0.02, 0.10, 0.87 and 0.01 in CB and 0, 0.09, 0.91 and 0 in AR; and those of *Me-1*, (*Rfs* of 100 and 88) were 0.91 and 0.09 in CB, 0.95 and 0.05 in MON, and 0.92 and 0.08 in PM.

The genotypic frequencies of polymorphic loci in all populations were in Hardy-Weinberg equilibrium. Where replicate samples from the same locations (CB) were analyzed, contigency χ^2 values were insignificant, and therefore data for replicate sets were pooled. Statistical analysis of genotypic frequency data showed that there was more variability in the northern populations (Tables 1 and 2), with 55% (range 48–60%) polymorphic loci, mean heterozygosity of 20.4% (19.8–21.4%) and a mean number of 3.0 (2.6–3.6) alleles per locus; the values for southern populations were 24% (16–40), 9.1% (5.3–15.5) and 1.45% (1.3–2.7). Almost all southern populations ranked lower in mean heterozygosity than northern populations with respect to the 5 most variable loci: *Had-1*, *6Pgd-1*, *Acon-2*, *Pgm-1* and *Mdh-1* (Fig. 2).

Although no diagnostic allelic differences were observed among northern and southern populations, there were differences for frequencies of electromorphs at certain loci. Most southern populations were fixed or nearly fixed (freq. of most common allele 0.95 or more) for the Had-1^{82} allele, which occurred at frequencies of 0.54 in CB and Mon and 0.71 in AR. *Est-*4^{95} allele was the most common allele in northern (freq. 0.81, 0.73 and 0.58 in AR, CB and Mon, respectively), but was either not observed (in the PM and ICA populations) or occurred at low frequencies (0.05 in PER, 0.08 in PB, 0.10 in SAL) in most southern populations (Table 1). The allele *Est-*6^{94}, which was common (freq. 0.27–0.37) in the north, was not observed in 7 of the 8 southern populations (Table 1). For *6Pgd-1* and *Had-1*, northern populations showed alleles twice or higher in number than the southern populations. In addition, certain alleles (*Est2*^{103}; *Pep-*4^{180}; *Rfs*, 146, 100 and 86 of *Pgm-1*; *6Pgd-*1^{124} and *Acon-*1^{92}) were present in northern populations, but were not observed in most southern populations.

Certain alleles were more common in the south. For example, the frequency of *Est-*6^{100} in most southern populations (SAL, PM and PER) was about twice that observed in northern populations. The distribution of some electromorphs was patchy among 8 southern populations. For example, *Pep-*4^{46} was present in SAL but absent in others; *EST-2 Rfs*, 95 and 97, occurred at frequencies of 0.12 and 0.19 in PM and 0 and 0.10 in PB, respectively, but was absent in others; *Est-8 Rf*, 100, was present in PER (freq. 0.27) and PB (freq. 0.24) but absent in PIZ, ORP , and ICA; *Sodh Rf*, 79, was present only in PB but absent in others; *Xdh-2 Rf*, 88, was present in PER (freq. 0.17) and SAL (freq. 0.11) but absent in ORP, PM and ICA; and *Xdh-2 Rf*, 117, was present in PM but absent in almost all others. In addition, of the 8 southern populations, PB revealed maximum number of alleles at 8 loci (*Acon-1*, *Ao-1*, *Est-4*, *Pep-4*, *6Pgd*, *Pgm-1*, *Sodh* and *Xdh-2*).

Table 3 lists values of genetic similarities (I) and differences (D) (Nei 1978) among populations. Northern populations were relatively more closely related among themselves (I =

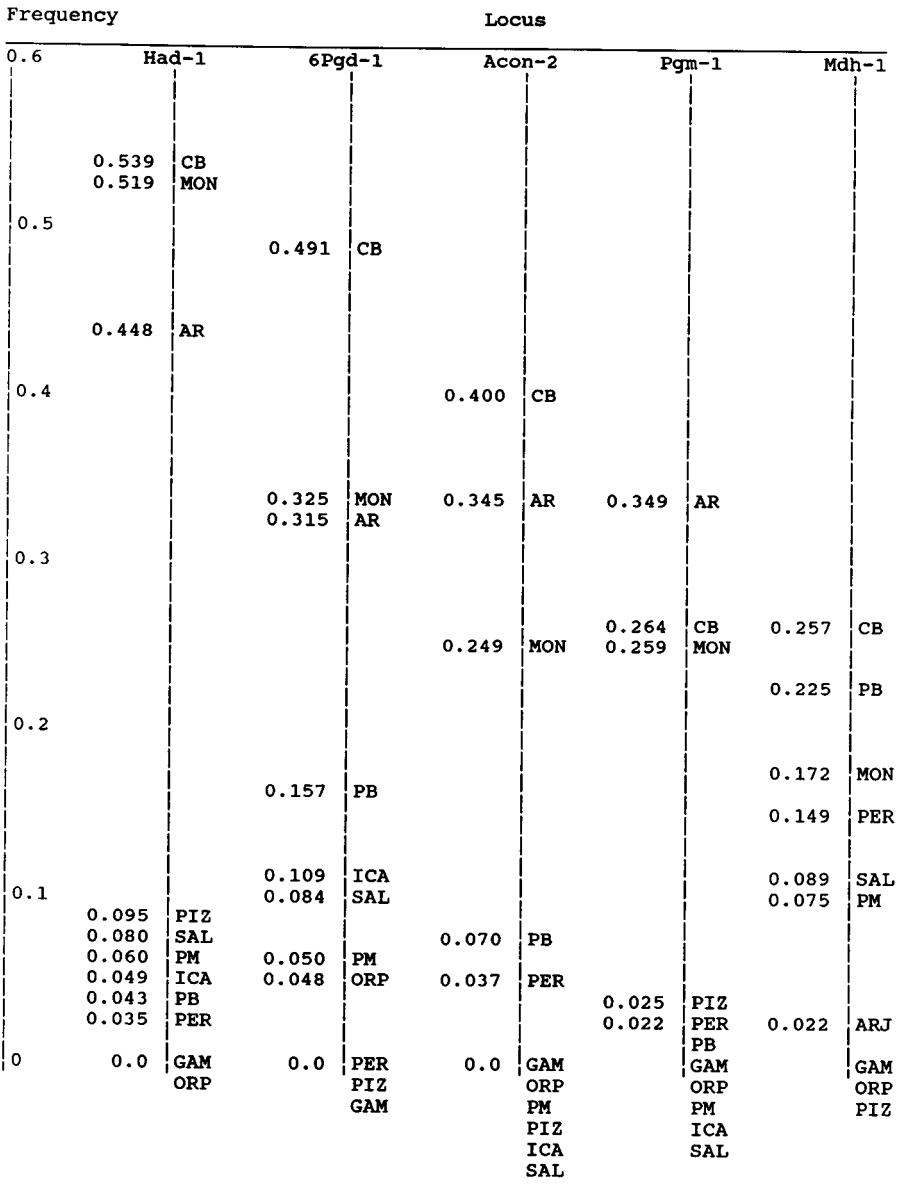

Fig. 2. A schematic representation of mean heterozygosity levels in northern and southern populations of *Anopheles albimanus* from Colombia. Refer to Materials and Methods section for key to abbreviations.

0.989–0.996) than they were to southern populations (I = 0.950–0.971). A phenogram of genetic relationship (Fig. 3) using unweighted pair-group (with arithmetic means) clustering of Nei's (1978) distances showed the grouping of northern populations into a cluster distinct from that of southern populations, which was further divided into 3 subclusters. A similar relationship among geographical populations was observed when modified Roger's distance (Wright 1978) was used. The cladistic relationships (based on the Wagner procedure using Roger's distance matrices) among populations was similar to the genetic relationships shown in Fig. 3.

Chromosomal patterns: Comparison of larval salivary gland chromosomes in AR ($n = 51$), CB ($n = 33$), MON ($n = 22$), GAM ($n = 23$), PB ($n = 18$), ICA ($n = 27$), PER ($n = 18$), SAL ($n = 16$) and Padula, near the AR collection site ($n = 8$), revealed no difference in banding patterns. Chromosome complements in the hybrids from crosses between field strains and BM-1 showed

Table 3. Estimates of Nei's (1978) unbiased genetic identity (above diagonal) and genetic distance (below diagonal) for natural populations of *Anopheles albimanus*.

Population	CB	MON	AR	GAM	ORP	PM	PIZ	ICA	PER	PB	SAL
CB	—	0.992	0.996	0.968	0.966	0.951	0.959	0.950	0.951	0.961	0.951
MON	0.008	—	0.989	0.962	0.962	0.955	0.957	0.952	0.953	0.956	0.951
AR	0.004	0.011	—	0.971	0.968	0.953	0.961	0.951	0.952	0.960	0.952
GAM	0.033	0.039	0.030	—	0.983	0.969	0.970	0.965	0.973	0.986	0.969
ORP	0.034	0.038	0.033	0.017	—	0.990	0.995	0.991	0.989	0.986	0.990
PM	0.051	0.046	0.048	0.031	0.010	—	0.992	0.992	0.993	0.976	0.989
PIZ	0.042	0.044	0.040	0.030	0.005	0.008	—	0.996	0.994	0.971	0.996
ICA	0.051	0.050	0.050	0.036	0.009	0.008	0.004	—	0.994	0.971	0.992
PER	0.050	0.048	0.049	0.028	0.011	0.007	0.006	0.006	—	0.977	0.996
PB	0.040	0.044	0.041	0.014	0.015	0.025	0.030	0.030	0.024	—	0.974
SAL	0.051	0.050	0.049	0.031	0.010	0.011	0.004	0.008	0.004	0.026	—

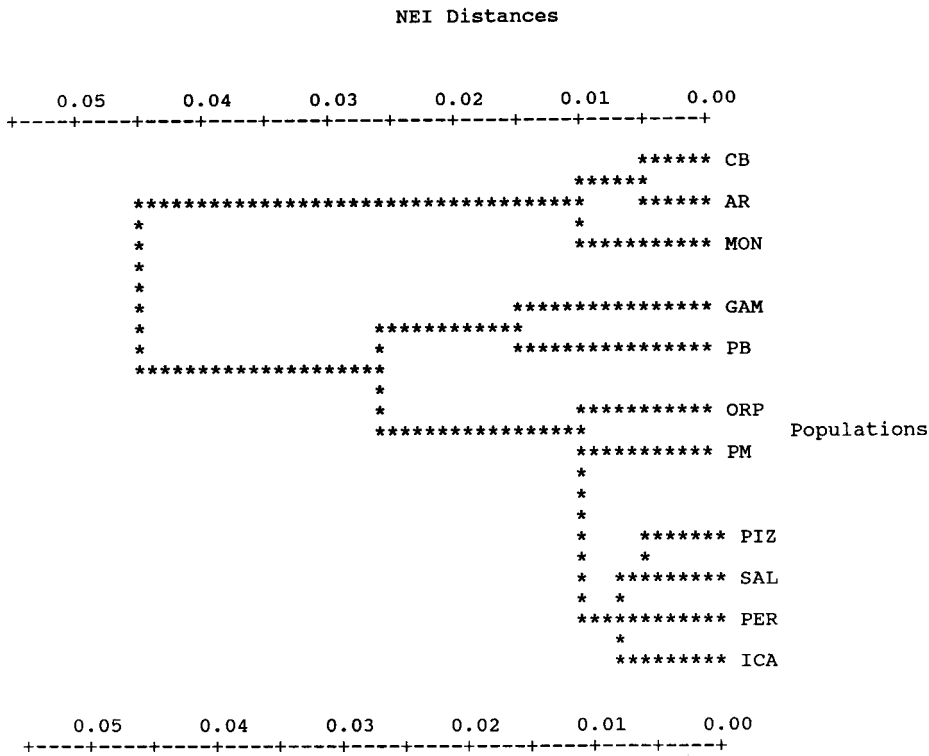

Fig. 3. Phenogram of genetic relationships among northern and southern populations of *Anopheles albimanus* from Colombia using unweighted pair-group (with arithmetic means) clustering of Nei (1978) distances. Refer to Materials and Methods section for key to abbreviations.

complete synapsis. Only one of the 51 preparations from CB revealed a short asynapsis near the proximal end (possible inversion) of the X chromosome.

Hybridization crosses: All parental and backcrosses resulted in hatch and survival comparable to control groups (BM-1 or INS strains). Parental crosses (in both directions) between INS and CB, INS × AR, INS × GAM, INS × TUMACO and INS × BM-1 resulted in high percent hatch (± SD) ranging from 82.5 ± 13.5 (INS × GAM) to 91.3 ± 9.7 (INS × AR). The controls, INS × INS and BM-1 × BM-1 produced 78.1 ± 8.9 and 88.7 ± 11.9, respectively. The hybrid males and females were backcrossed to BM-1 strain. The percent hatches (± SD) were 80.9 ± 13.7 (INS/CB × BM-1), 79.1 ± 11.4 (BM-1 × INS/CB), 78.3 ± 15.6 (INS/AR × BM-1) and 89.5 ± 9.7 (BM-1 × INS/AR). In some backcrosses only hybrid males were used. The percent hatches were 87.9 ± 12.3 (BM-1 × INS/GAM) and 84.1 ± 11.1 (BM-1 × INS/TU-

MACO), compared with 87.1 ± 9.9 for control strains (BM-1 × BM-1/INS). These results showed that the hybrid males and females were fertile (results of backcrosses) and had normal testes and ovaries, respectively.

DISCUSSION

The southern populations displayed lower levels of variability than northern populations (Table 2). This can be attributed to small population sizes and narrow habitats provided by the estuaries, which are ideal for promoting inbreeding resulting in random genetic drift or marginality effect and selection. Lack of diagnostic loci, chromosomal differences and hybrid sterility in cross matings among geographical populations indicate the absence of cryptic speciation in populations of *An. albimanus* in Colombia. Previous cytogenetic studies on natural populations from Panama, El Salvador, Mexico, Haiti and the Dominican Republic in our laboratory (Gainesville) also failed to reveal species level genetic differentiation (data not shown).

The level of variability in the northern populations (Table 2) was typical of many North American mosquitoes (Hilburn et al. 1984, Narang et al. 1989b) and South American anopheline species of the subgenus *Nyssorhynchus* (Narang 1980; Narang et al. 1979a, 1979b). For example, wild populations of the *An. quadrimaculatus* Say species complex displayed polymorphism at 50–73% of its loci, a mean heterozygosity of 0.18–0.23 per locus and a mean number of 2.80–3.43 alleles per locus (Narang et al. 1989b). The relatively lower variability in the coastal saltwater population (MON) as compared with freshwater populations of *An. albimanus* is in agreement with similar studies conducted on other South American anophelines, the saltwater breeder *An. aquasalis* Curry and freshwater breeders *An. albitarsis* Lynch Arribalzaga and *An. argyritarsis* Robineau-Desvoidy (Narang 1980). Except for the GAM population which was collected from an abandoned shrimp breeding pond, the remaining 7 southern populations were from saltwater habitats. One explanation for this difference in variability would be that the constraints in the saltwater habitats result in a narrow niche which promotes inbreeding and genetic drift. It is also possible that the salt water niche might be marginal at best and select for a more specialized genotype (Carson 1959, Carson and Kaneshiro 1976, Prakash 1973).

Lack of significant chromosomal and allozyme differentiation among geographical populations of *An. albimanus* is in sharp contrast to high degrees of chromosomal (Kitzmiller 1976, 1977) and allozyme (Steiner et al. 1982) differentiation in other neotropical anophelines of the subgenus *Nyssorhynchus* (*An. albitarsis*, *An. nuneztovari* Gabaldon and *An. aquasalis*) (Narang and Seawright 1990). Similarly, genetic studies on *An. gambiae* Giles and *An. maculipennis* Meigen showed that each species is in fact a species complex. Sibling species of *An. gambiae* differ considerably in their genetic makeup (allozyme frequencies) and their competence as malaria vectors (Mahon et al. 1976, White 1974, World Health Organization 1977). Recently, electrophoretic, chromosomal and hybridization studies on wild populations of *An. quadrimaculatus* Say led to the identification of 4 sibling species A, B, C and D (Kaiser 1988, Lanzaro et al. 1988; Narang et al. 1989a, 1989b, 1989c). The lack of significant intraspecific differentiation in *An. albimanus* as demonstrated in this paper indicates that these 11 natural populations represent conspecific populations (Ayala et al. 1975) of a single species.

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