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Mitochondrial DNA Divergence between Wild and Laboratory Populations of *Anopheles albimanus* Wiedemann (Diptera: Culicidae)

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Divergencia del ADN Mitocondrial entre Poblaciones del Campo y de Colonia de *Anopheles albimanus* Wiedemann (Diptera: Culicidae)

RESUMEN - Las colonias de laboratorio facilitan el estudio de los insectos vectores; sin embargo, se ha sugerido que tales colonias de insectos no son representativas de las poblaciones naturales, llevando en algunos casos, a interpretaciones erróneas respecto a la variación intraespecífica entre los individuos. En el presente estudio se evaluó la variabilidad del gen mitocondrial citocromo *b* entre una colonia de laboratorio de *Anopheles albimanus* Wiedemann fundada hace 20 años y la población del campo de la cual se derivó. Los análisis revelan la presencia de cinco y tres haplotipos en las poblaciones del campo y la colonia, respectivamente; los individuos del campo presentaron una mayor variabilidad que los de la colonia basada en el numero de sitios polimórficos, la diversidad haplotípica, la diversidad nucleotídica y el valor promedio de las diferencias nucleotídicas. El promedio y el número neto de sustituciones nucleotídicas por sitio entre las poblaciones y los valores calculados para el F_{ST} (0,37179, P = 0.05) indican que existe un considerable grado de diferenciación genética entre ellas; el árbol filogenético muestra que los haplotipos de la colonia parecen derivarse de las poblaciones del campo. Estos resultados sugieren una mayor variabilidad genética existente entre los especimenes del campo en comparación con los individuos de la colonia debido en parte, al largo tiempo de colonización.

PALABRAS-CLAVE: Variabilidad genética, malaria, mosquito

ABSTRACT - Studies of insect vectors may be facilitated by using laboratory colonies. However, it has been suggested that the colony insects are not representative of natural populations, sometimes yealding to erroneous interpretations of the intraspecific genetic variation between the individuals. In the present study the variability of the mitochondrial gene cytochrome *b* was evaluated among a closed laboratory colony of *Anopheles albimanus* that was founded 20 years ago and the field population from which it was derived. The analyses revealed the presence of five and three nucleotide haplotypes in the wild and colony populations, respectively. Wild individuals presented greater variability than those of the colony based on the number of polymorphic sites, haplotype diversity, nucleotide diversity and mean values of nucleotide differences. The mean and net numbers of nucleotide substitutions per site between populations and the significant F_{ST} value calculated (0,37179, P = 0.05) indicate that there is a considerable degree of genetic differentiation between them. The phylogenetic tree showed that the colony haplotypes appear to be derived from the wild population. These results suggest a great genetic variability in wild specimens compared with the laboratory ones as a consequence of a long time of colonization.

KEY WORDS: Genetic variability, malaria, mosquito

Anopheles albimanus Wiedemann, is one of the principal malaria vectors in South and Central America. Because of its medical importance this species has been the focus of numerous biological, bionomic and systematic studies (Frederickson *et al*. 1992). Such studies require either observations of the mosquitoes in their natural habitat or the establishment of laboratory colonies, the latter permitting immediate access to the insects and facilitating the control and follow-up of the individuals analysed.

Although many entomological studies benefit from the use of laboratory colonies, it has been suggested that these might not be representative of natural populations (Mukhopadhyay *et al*. 1997). Among other reasons, this could be due to the fact that individuals colonised over long periods tend to reduce their genetic and phenotypic variability, sometimes increasing the frequency of rare alleles and abnormal genotypes that habitually present low productivity in the natural population (Munstermann *et al*. 1994, Mukhopadhyay *et al*. 1997, Norris *et al*. 2001). On the other hand bottleneck phenomena may be presented that give rise to the founder effect (Matthews & Craig 1987). In contrast, it has been found that the wild population preserve more genetic variability than the colonies. This fact is greatly favoured by behaviours, like migration, that occur in the nature and permits a major gene flow between the individuals of the same specie (Futuyma 1998).

Genetic variability at the molecular level is measured as mutation rate; this fact is possible making a comparison of the DNA sequences from a specific marker. The objective of the present study was to evaluate the genetic variability of the mitochondrial gene cytochrome *b* from a natural population of *An. albimanus*, and make a comparison with the genetic variability showed by a laboratory colony found with the same species twenty years ago.

Material and Methods

Collection of Specimens. Eight specimens of *An. albimanus* were collected in the municipality of Santa Rosa de Lima (10°26'N, 75°22'W, 50 m.a.s.l.) in the Department of Bolívar on the Caribbean coast of Colombia. The specimens were captured on human bait and with CDC light traps and Shannon traps. The mosquitoes were subsequently transported to the laboratory in 100% isopropanol and identified to species using the dichotomous key of Suárez *et al*. (1988). In addition, seven specimens were obtained from a closed colony of *An. albimanus* established in the Programa de Estudio y Control de Enfermedades Tropicales (PECET) at the University of Antioquia and founded from specimens captured in Santa Rosa de Lima 20 years before.

Extraction and Amplification of DNA. To extract genomic DNA 100µl of grind buffer (0.06 M EDTA, 0.1 M Tris-HCL, 0.08 M NaCl, 0.16 M Sucrose, 5% SDS) were used, following the methodology of Collins *et al*. (1987). The primers used for PCR were CB3FC CA(T/C)ATTCAACC(A/T)GAATGATA and NINFR GGTA(C/T)(A/T)TGCCTCGA(T/A)TTCG(T/A)TATGA. These amplify the 3' extremity of the cytochrome *b* gene, all the tRNA for serine and the 3' extremity of gene subunit one of the NADH dehydrogenase. The reaction mix consisted of 0.5 µl of the DNA extract solution (approximately 2 ng/µl), 60 µM of each deoxynucleotide (Promega Corp., Madison, WI), 0.002 M of each primer, 5 µl of buffer without $MgCl_2$ (Promega), 4 mM of $MgCl_2$, and ultrapure sterile water adjusted to a final volume of 50 µl.

PCR was carried out in a Perkin Elmer thermocycler under the following thermic profile: an initial denaturation step at 94°C for 3 min, followed by 35 denaturation cycles at 93°C for 1 min, alignment at 50°C for 1 min and extension at 72°C for 1 m, terminating with an extension step at 72°C for 10 min. The PCR products were visualised by means of electrophoresis in 1% agarose gel, previously stained with ethidium bromide. Each of the amplification products was purified with the Wizard PCR Preps DNA Purification System Kit by Promega, following the manufacturer's instructions.

Procurement and Analyses of the Sequences. PCR products were directly sequenced in the forward and reverse directions on an ABI 3700 Capillary DNA sequencer using Big Dye fluorescent terminators (Big Dye cycle sequencing kit version 2; Perkin Elmer). The sequences obtained were edited using the SeqMan 3.03 programme (DNAStar, Inc) and aligned with homologous sequences of *An. gambiae* Giles (1902) (Beard et al. *1993*, L20934) and *An. quadrimaculatus* Say (1824) (Mitchell *et al*. 1993, L04272) using the CLUSTAL W 1.7 programme (Thompson *et al*. 1994) incorporated in DAMBE 4.0.41 software (Xia & Xie 2001). The new sequences generated for *An. albimanus* have been deposited in Genbank under the following accession numbers: AY542138-AY542151. Basic sequence statistics were calculated using MEGA 2.1 (Kumar *et al*. 2001).

The following parameters were used to estimate genetic variability between the wild and colony populations of *An. albimanus*: number of polymorphic sites (*S*), haplotype diversity (*h*) (Nei 1987), nucleotide diversity (*Pi*) (Lynch & Crease 1990) using the Jukes and Cantor correction (Jukes & Cantor 1969), mean number of nucleotide differences (*k*) (Tajima 1983), mean number of nucleotide substitutions per site between populations (*Dxy*) (Nei 1987) using the Jukes and Cantor method (Jukes & Cantor 1969), number of net nucleotide substitutions per site between populations (*Da*) (Nei 1987) with the Jukes and Cantor correction (Jukes & Cantor 1969), and extent of genetic differentiation between the populations (F_{ST}) (Hudson *et al*. 1992).

These parameters were obtained with the DnaSP programme version 3 (Rozas & Rozas 1999). The same programme was used to carry out the Tajima Test (1989) (*D*) that permits determination of the neutrality of the nucleotide changes encountered.

A maximum likelihood dendrogram was produced via heuristic search (10 random replicate addition searches with tree-bisection-reconnection branch swapping) by PAUP* 4.0b10 for Macintosh (Swofford 2002). Transition/transversion ratios were estimated for the substitution model and a discrete approximation to gamma distribution was estimated for among-site rate variation. Default settings were

maintained for all other options, yielding the equivalent of the Hasegawa-Kishino-Yano model (Hasegawa *et al*. 1985). Bootstrap support (Felsenstein 1985) was estimated by a heuristic search on 100 bootstrap pseudoreplicates, each with 10 random additions and TBR branch swapping. Both *An. gambiae* and *An. quadrimaculatus* were selected as outgroups for these analyses. The obtained tree was visualised by Treeview 1.6.6 (Page 1996).

Results and Discussion

The values obtained for the parameters of genetic diversity *S*, *h*, *Pi*, *k*, *Dxy*, and *Da*, clearly indicate that wild individuals possess greater genetic variability than those of the colony. The fragment resulting was a 222 bp in lenght corresponding to the 3' extremity of the cytochrome *b* gene, it was obtained from seven wild individuals and seven from the colony. This is the first time that this portion of the gene has been sequenced for *An. albimanus*. The fragment is located between positions 11328 and 11549 of the *An. gambiae* mitochondrial genome (Beard *et al*. 1993). Additionally, sequences were obtained for tRNA for serine (65 bp), intergenic spacer one (17 bp) and the 3' extremity of gene subunit one of NADH dehydrogenase (148 bp). However, these latter were excluded from the analyses due to their very low polymorphism or to the lack of information for all the individuals. Consequently, analyses concentrated on the mitochondrial region that coded for cytochrome *b* protein. This region has been used in molecular phylogenetic studies of insects (Simmons & Weller 2001).

The nucleotide composition of the wild population was represented by A (33.8%), T (38.6%), G (12.6%) and C (15.0%), and that of the laboratory colony by A (34.2%), T (39.4%), G (12.2%) and C (14.2%). Alignment of the nucleotide sequences of the cytochrome *b* gene of *An. albimanus* with homologous sequences of *An. gambiae* and *An. quadrimaculatus* is shown in Fig. 1. In all 52 (23.4%) of

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                          111 111 111 122 222 222 223 333 333 333 444 444 444
                  123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678
A. gambiae        CCT TTC ACA CAC TCT AGC AAG TTT CGA GGA TTA CAA TTT TAC CCA TTA
A. quadrimaculatus ... ..T ..T ..T ..A ..T ..A ..C ... ... C.C ... ... ... ..T ...
A. albimanus Wild1 ... ..T ... ... ..A ..T ... ..C ... ..C AC. ... ... ... ... ...
A. albimanus Wild2 ... ..T ... ... ..A ..T .TA ..C ... ... ... ... ... ... ... ...
A. albimanus Wild3 ... ..T ... ... ..A ..T .TA ..C ... ... ... ... ... ... ... ...
A. albimanus Wild4 ... ..T ... ... ..A ..T ..A ..C ... ... ... ..G ... ... ... ...
A. albimanus Wild5 ... ..T ... ... ..A ..T ..A ..C ... ... ... ..G ... ... ... ...
A. albimanus Wild6 ... ..T ... ... ..A ..T ..A ..C ... ... ... ..G ... ... ... ...
A. albimanus Wild7 ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...
A. albimanus Lab1  ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...
A. albimanus Lab2  ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...
A. albimanus Lab3  ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...
A. albimanus Lab4  ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...
A. albimanus Lab5  ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...
A. albimanus Lab6  ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...
A. albimanus Lab7  ... ..T ... ... ..A ..T ..A ..C ... ... ... ... ... ... ... ...

                  455 555 555 556 666 666 666 777 777 777 788 888 888 889 999 999
                  901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456
A. gambiae        AAT CAA ATT TTA TTC TGA AAT ATA GTA ATT GTA GCT TCG TTA TTA ACT
A. quadrimaculatus ..C ... ... ... ..T ... ... ... ... G.A ... ... ..A ... ... ...
A. albimanus Wild1 ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Wild2 ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Wild3 ... ... ... ... .A. ... ... ... .G. ... ... ... ... ... ... ..A
A. albimanus Wild4 ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Wild5 ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Wild6 ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Wild7 ... ... ... ... .A. ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Lab1  ... ... ... ... .AT ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Lab2  ... ... ... ... .AT ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Lab3  ... ... ... ... .AT ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Lab4  ... ... ... ... .AT ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Lab5  ... ... ... ... .AT ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Lab6  ... ... ... ... .AT ... ... ... ... ... ... ... ... ... ... ..A
A. albimanus Lab7  ... ... ... ... .AT ... ... ... ... ... ... ... .GC ... ... ..A
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Figure 1. Alignment of the cytochrome *b* nucleotide sequences in wild and laboratory populations of *An. albimanus*. The dots represent the same nucleotide as occurs in the outgroup species *An. gambiae* (L20934).

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                      111 111 111 111 111 111 111 111 111 111 111 111 111 111 111
                  999 000 000 000 011 111 111 112 222 222 222 333 333 333 344 444
                  789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234
A. gambiae        TGA ATT GGA GCT CGA CCA GTA GAA GAC CCA TAT ATT TTA ACA GGT CAA
A. quadrimaculatus ... ... ... ..C ... ... ..T ... ... ..T ... G.A ... ... ..G ...
A. albimanus Wild1 ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..G ...
A. albimanus Wild2 ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Wild3 ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Wild4 ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Wild5 ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Wild6 ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Wild7 ... ... ... ... ..T ..G ... ... ... ..T ... ... ... ..T ..G ...
A. albimanus Lab1  ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Lab1  ... ... ..G ... ..T ..G ... ... ..T ..T ... ... ... ..T ..A ...
A. albimanus Lab2  ... ... ..G ... ..T ..G ... ... ..T ..T ... ... ... ..T ..A ...
A. albimanus Lab3  ... ... ..G ... ..T ..G ... ... ..T ..T ... ... ... ..T ..A ...
A. albimanus Lab4  ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Lab5  ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...
A. albimanus Lab6  ... ... ..G ... ..T ..G ... ... ... ..T ... ... ... ..T ..A ...

                  111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111
                  444 445 555 555 555 666 666 666 677 777 777 778 888 888 888 999
                  567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012
A. gambiae        ATT TTA ACT GTA TTA TAT TTC TCT TAC TTT ATT ATT AAT CCT TTA TTA
A. quadrimaculatus ... ... ..A ... ... ..C ..T ... ..T ... ... ... ... ... ..G ...
A. albimanus Wild1 ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Wild2 ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Wild3 ... C.G ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Wild4 ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Wild5 ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Wild6 ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Wild7 ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Lab1  ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Lab2  ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Lab3  ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Lab4  ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Lab5  ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Lab6  ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T
A. albimanus Lab7  ... C.T ..A ... ... ... ..T ..A ... ... ... ... ... ..A ... G.T

                  111 111 122 222 222 222 222 222 222 222
                  999 999 900 000 000 001 111 111 111 222
                  345 678 901 234 567 890 123 456 789 012
A. gambiae        GCA AAG TTT TGA GAT AAG CTA TTA AAT TAA
A. quadrimaculatus ..T ..A .AC ... ... ..A T.. C.. ... ...
A. albimanus Wild1 ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Wild2 ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Wild3 ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Wild4 ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Wild5 ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Wild6 ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Wild7 ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Lab1  ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Lab2  ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Lab3  ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Lab4  ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Lab5  ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Lab6  ..T ... .A. ... ... ..A T.. ... ... ...
A. albimanus Lab7  ..T ... .A. ... ... ..A T.. ... ... ...
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Figure 1. Continuation.

the 222 base sites were variable and 170 (76.6%) were constant. When the sequences of *An. gambiae* and *An. quadrimaculatus* are excluded, the number of monomorphic sites was 208 (93.7%) and that of polymorphic sites 14 (6.31%) (Fig. 1). Only one of the latter was shared between the two *An. albimanus* populations (position 63 of the alignment, Fig. 1). Ten of the polymorphic sites appeared among the field specimens (positions 20, 21, 30, 31, 32, 36, 74, 105, 141 and 150 of the alignment, Fig. 1), while only three sites (positions 86, 87 and 123 of the alignment, Fig. 1) were presented within the colony insects. This means that nucleotide diversity and the mean number of nucleotide differences were three times greater in the wild population than in the laboratory colony. Most of the changes were A–T tranversions (50%), as commonly occurs in mitochondrial genes that code for proteins in insects (Simon *et al*. 1994). The mean and net numbers (±1SD) of nucleotide substitutions per site between populations were 0.01696 (±0.00442) and 0.00631 (±0.00439), respectively. The nucleotide diversity was 0.01611 in the wild population and 0.00518 in the laboratory colony. The mean numbers of nucleotide differences of the wild and laboratory populations were 3.524, and 1.143, respectively (Table 1).

These substitutions defined eight nucleotide haplotypes, five of them in the wild population and three in the laboratory colony. As a reflection of the high frequency of haplotypes in the wild population, haplotype diversity was greater in this group (0.857) than in the colony insects (0.714) (Table 1). After translation of amino acids, the number of haplotypes continued to be high in the field population with four haplotypes compared to only two in the colony population (Fig. 2). Four the nucleotide substitutions of the wild

Table 1. Genetic diversity of the cytochrome *b* gene in wild and laboratory populations of *An. albimanus*.

Values	Colony	Wild
Polymorphic sites	3	13
Parsimoniously informative sites	1	4
Haplotypes	3	6
Haplotype diversity	0.714	0.893
Mean number of nucleotide differences, K	1.14286	4.10714
Nucleotide diversity, with Jukes & Cantor correction	0.00520	0.01890
Tajima test (D)	-0.30187	-0.91566

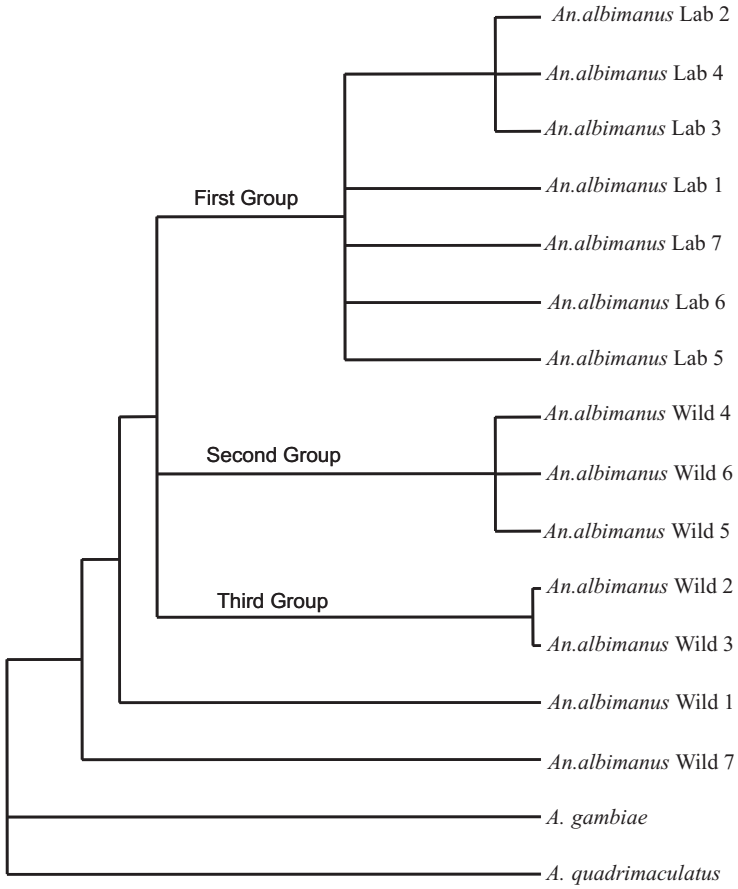

Figure 2. Tree of maximum probability obtained with PAUP estimated transition/transversion ratio. The colony population forms a monophyletic group (first group) supported by a bootstrap value of 62, and within the wild group. Although, some specimens of wild populations forms the second and third group all of them as a part of this big wild population group.

Tabla 2. Variation of genetic distance (HKY) values between wild and laboratory populations (ingroup), including *An. gambie* and *An. quadrimaculatus* (outgroups). Genetic distances varied from 0 to 0.01365 in the laboratory population, and 0 to 0.03700 in the wild insects.

HKY85 Distance Matrix																
Individuals	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *An. gambiae*																
2 *An. quadrimaculatus*	0.17171	-														
3 Wild1	0.13373	0.18069	-													
4 Wild2	0.12844	0.17532	0.02760	-												
5 Wild3	0.13395	0.18142	0.03700	0.00908	-											
6 Wild4	0.12860	0.17585	0.02768	0.00907	0.01825	-										
7 Wild5	0.12860	0.17585	0.02768	0.00907	0.01825	0.00000	-									
8 Wild6	0.12860	0.17585	0.02768	0.00907	0.01825	0.00000	0.00000	-								
9 Wild7	0.11762	0.15751	0.02295	0.01368	0.02290	0.01375	0.01375	0.01375	-							
10 Laboratory1	0.12866	0.16362	0.02768	0.00907	0.01826	0.00911	0.00911	0.00911	0.01375	-						
11 Laboratory2	0.13432	0.16984	0.03248	0.01368	0.02290	0.01375	0.01375	0.01375	0.01845	0.00453	-					
12 Laboratory3	0.13432	0.16984	0.03248	0.01368	0.02290	0.01375	0.01375	0.01375	0.01845	0.00453	0.00000	-				
13 Laboratory4	0.13432	0.16984	0.03248	0.01368	0.02290	0.01375	0.01375	0.01375	0.01845	0.00453	0.00000	0.0000	-			
14 Laboratory5	0.12866	0.16362	0.02768	0.00907	0.01826	0.00911	0.00911	0.00911	0.01375	0.00000	0.00453	0.00453	0.00453	-		
15 Laboratory6	0.12866	0.16362	0.02768	0.00907	0.01826	0.00911	0.00911	0.00911	0.01375	0.00000	0.00453	0.00453	0.00453	0.00000	-	
16 Laboratory7	0.13946	0.16871	0.03707	0.01826	0.02758	0.01827	0.01827	0.01827	0.02295	0.00908	0.01365	0.01365	0.01365	0.00908	0.00908	-

population were not silent (positions 20 [Lysine x Methionine], 31 and 32 [Leucine x Threonine], 74 [Valine x Glycine] of the alignment, Fig. 1), while in the colony only one was not silent (position 86 and 87 [Serine x Cysteine] of the alignment, Fig. 1). The values of the Tajima parameter *(D)* were –0.73333 ($P > 0.10$) for the field population and –0.30187 ($P > 0.10$) for the laboratory colony, so that selection can be discounted as being responsible for the diversity encountered (Table 1) and the F_{ST} value calculated between both populations was 0.37179 ($P = 0.05$), suggesting that there is a considerable degree of genetic differentiation between them. Similar values have been discovered when comparing divergent geographic populations in insect vectors of pathogens (Oliveira *et al*. 2001).

The analyses of maximum probability of the cytochrome *b* gene under the model HKY (Hasegawa *et al*. 1985) produced the tree shown in Fig. 3. This model incorporated frequency of unequal bases {$p_{(A)} = 0.333684$, $p_{(G)} = 0.121123$, $p_{(C)} = 0.149103$, $p_{(T)} = 0.396090$} and an estimated transition/transversion ratio (Ti/tv) of 1.267529. In the ML tree, the colony population forms a monophyletic group supported by a bootstrap value of 62. This monophyletic group is found contained within the group of the field population, suggesting that the colony individuals only contain a part of the variabiliy reflected by the wild populations to this gene; nevertheless, none of the nucleotide haplotypes was shared among the two *An. albimanus* populations. The bootstrap support values for the clades were relatively lows (55-67), with the exception of the clade *An. albimanus* bear grouping, which had a bootstrap value of 98%. The genetic distances (HKY) varied from 0 to 0.01365 in the colony population, and 0 to 0.03700 in the wild insects (Table 2). The maximum value within the ingroup was 0.03707, found when haplotypes Wild1 and Lab7 were compared.

The low genetic variation observed in the colony individuals could be explained by the phenomenon of genetic drift or founder effect, given that the changes in a conserved gene and the values of the Tajima *D* parameter eliminate the possibility that selection is responsible for this reduction in diversity. However, one cannot discount the possibility that the colony represents a less diverse population of 20 years ago and that the current population has greater genetic diversity as a consequence of the phenomenons of migration, resulting in a major flow of haplotypes between the wild populations. The latter is probable, considering that *An. albimanus* possesses a great fly capacity of up to 10 km and is widely distributed along the Caribbean and Pacific coasts of Colombia (Frederickson 1992).

Independently of the phenomena that explain the observed differences, it is evident that the cytochrome *b* gene sequences obtained from colony individuals only reflect part of the genetic variation of the current natural population. These results agree with others found for isoenzymes and microsatellites in insects such as *An. gambiae* (Norris *et al*. 2001), *Aedes aegypti* L. (1762) (Munstermann 1994) and *Lutzomyia longipalpis* Lutz and Neiva (1912) (Mukhopadhyay *et al*. 1997). It has been shown that laboratory conditions could alter the population genetic structure of these vectors, giving rise to phenotypes and genotypes different from those encountered in the wild. The findings of this study suggest that certain care should be taken in making inferences from laboratory-reared insects with respect to natural populations when the cytochrome *b* gene is used in molecular systematic studies.

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