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Analysis of the genetic variability and structure of *Ochlerotatus taeniorhynchus* (Diptera: Culicidae) populations from the Colombian Atlantic coast on the basis of random amplified polymorphic DNA markers

F.J. Bello[1], N.A. Segura[1] and M. Ruiz-Garcia[2]

[1]Laboratory of Medical Entomology, School of Medicine and Health Sciences, Universidad del Rosario, Bogotá DC, Colombia
[2]Laboratory of Molecular Population Genetics and Evolutionary Biology, Genetic Unit, Department of Biology, Faculty of Sciences, Pontificia Universidad Javeriana, Bogotá DC, Colombia

Corresponding author: F.J. Bello
E-mail: felio.bello@urosario.edu.co

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ABSTRACT. *Ochlerotatus taeniorhynchus* (Diptera: Culicidae) is a mosquito, which is an efficient vector of the virus causing epidemic-epizootic Venezuelan equine encephalitis in Colombia. This study used 9 random amplified polymorphic DNA (RAPD) markers to analyze the mosquito's genetic variability and genetic structure of 122 specimens in 7 populations from the Colombian Atlantic coast. Assuming that all loci were in Hardy-Weinberg equilibrium, diversity statistics and analyses were performed. The average number of amplified fragments for each primer was 8.3, and the size of these fragments ranged from 350 to 3600 bp. The expected average heterozygosity was 0.358 ± 0.103. The genetic heterogeneity among the populations studied was small (G_{ST} =

0.05 ± 0.01); meanwhile, the gene flow estimates (N_m = 7.32 ± 1.35) were high. In an identical way, the Nei's genetic distances obtained yielded very small values amongst the populations that were studied in this Colombian region. Furthermore, a spatial autocorrelation analysis with Moran's I index revealed a very weak, or inexistent, spatial genetic structure among these populations. The comparison of these results with those performed for other markers (isoenzymes and microsatellites) in populations of this same mosquito from the Colombian Atlantic coast was discussed. The results of our RAPD analysis showed scarce genetic differentiation among the mosquito populations on the Colombian Atlantic coast, which was probably determined by high gene flow levels.

Key words: *Ochlerotatus taeniorhynchus*; Genetic heterogeneity; Random amplified polymorphic DNA-polymerase chain reaction; Genetic variability; Spatial autocorrelation

INTRODUCTION

Ochlerotatus (*Aedes*) *taeniorhynchus* (Wiedemann) (Diptera: Culicidae) is considered a natural vector of the virus causing epidemic-epizootic Venezuelan equine encephalitis (VEEV) in the Americas. Specifically, typical epizootic VEEV strains of the subtypes IAB and IC are efficiently transmitted by this mosquito (Weaver et al., 2004). Additionally, this insect has also been identified as a competent vector of other arboviruses such as the Saint Louis encephalitis virus (Nayar et al., 1986), Eastern equine encephalitis virus (Ortiz et al., 2003), and West Nile virus (Eastwood et al., 2013). Furthermore, *O. taeniorhynchus* plays a major role in the transmission of the dog heartworm (*Dirofilaria immitis*) in South and Central America (Manrique-Saide et al., 2010).

O. taeniorhynchus has also been implicated in major coastal VEEV outbreaks ranging from northern South America to Texas (Weaver et al., 2004). Periodic human epidemics and re-emerging VEEV equine epizooty have been presented in Colombia since 1938. The last report of an epidemic-epizooty occurred in 1995 in the Colombian-Venezuelan Guajira. Around 75,000 to 100,000 human cases were estimated (directly leading to 300 deaths), with 3000 patients having neurological complications. This mosquito was recorded as being the most abundant in the quoted area by epidemiological evidence. Moreover, isolates of the virus and susceptibility assays in the laboratory led to incriminating this mosquito in the transmission of the aforementioned pathology (Rivas et al., 1997).

On the other hand, *O. taeniorhynchus* has a broad geographical distribution on the American continent, particularly on the coasts; it extends from Massachusetts in the USA to Santa Catarina in southern Brazil on the Atlantic side, and from California to Peru on the Pacific coast. In addition, the mosquito has been recorded on both Colombian Atlantic and Pacific coasts (Forattini, 1965).

We previously carried out 2 studies on the polymorphism and genetic structure of *O. taeniorhynchus* in populations from the Colombian Atlantic coast. In the first study, an isoenzyme analysis (Bello and Ruiz-García, 2009) was undertaken; meanwhile, in the second study, microsatellite loci were employed (Bello and Becerra, 2009). The results of these studies demonstrated, in general, low genetic differentiation among the populations that were analyzed,

with relatively high levels of genetic variability and high values of gene flow.

In the current work, we extended our previous genetic analyses of this mosquito species with random amplified polymorphic DNA (RAPD) markers. RAPD-polymerase chain reaction (PCR) is a technique that generates markers by the amplification of random DNA segments with single primers of arbitrary nucleotide sequence (Williams et al., 1990). These molecular markers have been successfully used in genetic variability studies; they were applied to natural populations of different mosquito species including *Aedes albifasciatus* (de Sousa et al., 1999), *Aedes* (*Finlaya*) *japonicus* (Fonseca et al., 2001), *Aedes albopictus* (Ayres et al., 2002), *Anopheles nuñeztovari* (Posso et al., 2003), *Anopheles superpictus* and *Anopheles pulcherrimus* (Abramova et al., 2005), *Anopheles darlingi* (González et al., 2007), and *Aedes aegypti* (Julio et al., 2009). However, there are no reports of studies performed on the genetics of *O. taeniorhynchus* populations on the Colombian Atlantic coast using these types of markers.

It is important to compare the population genetics results of these three types of markers (isoenzymes, microsatellites, and RAPD) because isoenzymes are believed to be more affected by natural selection because they are coding genes (structural), whereas microsatellites and RAPD are more neutral. Although microsatellites are widely dispersed in non-coding parts of the eukaryotic genome (Kashi et al., 1997), they could have an important role in the function and the regulation of the genome. Kashi and Soller (1999) and King and Soller (1999) showed that microsatellites could help individual genes and genomes in being equipped with adjustable "turning knobs." For instance, Kashi and Soller (1999) concluded that many microsatellites are functionally integrated within the genome; therefore, any changes in tract length can exert a quantitative regulatory effect on gene transcription activity and thus in adaptative potential. They showed that tandem repeats controlled the transcription activity of several genes in *Drosophila* (ultrabithorax, *hsp26*, and actin5C) and rats (prolactin gene). Additionally, Li et al. (2000) showed that edaphic factors may affect microsatellite variation in wild emmer wheat, either directly (selection) or indirectly (influencing mutational mechanisms or hitchhiking). In contrast, RAPDs are believed to fall within non-coding DNA, and they are not likely to be strongly linked to structural genes (Williams et al., 1990).

Thus, it is remarkably important to comparatively analyze the genetic structure and spatial genetic structure of these different markers among the same Colombian Atlantic *O. taeniorhynchus* populations because each molecular marker could be exposed to different evolutionary forces.

The main objectives of this study are as follows. First, we aimed to determine the levels of gene diversity in populations of *O. taeniorhynchus* on the Colombian Atlantic coast by means of RAPDs. Second, we estimated the degree of genetic heterogeneity and the gene flow among these mosquito populations using these same markers. Third, we aimed to determine the possible spatial genetic structure of these populations with the selected markers. Fourth, we compared the population genetics results that were obtained with RAPDs with those that were previously obtained with isoenzymes and microsatellites.

MATERIAL AND METHODS

Mosquito sampling sites, collection, and identification

Seven *O. taeniorhynchus* populations were analyzed. Adult mosquito specimens were

collected in the following cities and departments on the Colombian Atlantic coast: San Bernardo del Viento (09°22'22" latitude N and 75°57'21" longitude W), Coveñas (09°31'41" latitude N and 75°34'55" longitude W), Cartagena (10°23'59" latitude N and 75°30'52" longitude W), Barranquilla (10°57'42" latitude N and 74°46'54" longitude W), Ciénega (11°00'34" latitude N and 74°15'15" longitude W), Dibulla (11°17'00" latitude N and 73°19'00" longitude W), and Riohacha (11°33'59" latitude N and 72°54'37" longitude W) (Figure 1). Mosquitoes were collected during May, July, and August 2007. They were frozen and deposited in a tank of liquid nitrogen for transport to the Pontificia Universidad Javeriana's population genetics laboratory in Bogota, where they were kept in a freezer at -70°C until use in DNA extraction. Each specimen was identified using the taxonomic keys described by Nielsen (1983).

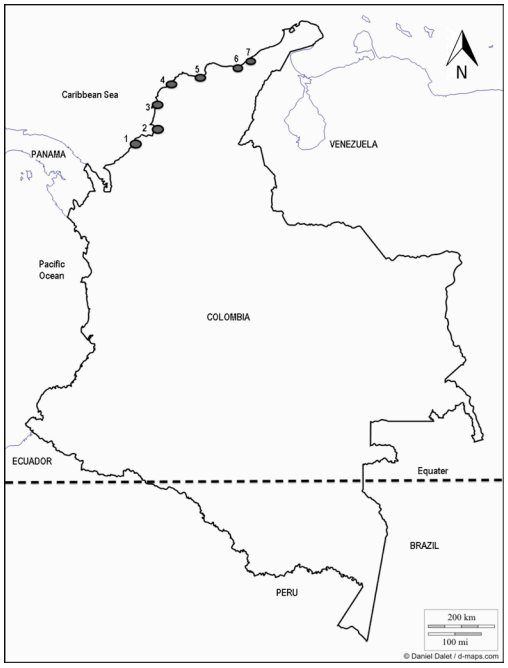

Figure 1. Collection sites for seven *Ochlerotatus taeniorhynchus* populations from the Colombian Atlantic coast. 1 = San Bernardo del Viento; 2 = Coveñas; 3 = Cartagena; 4 = Barranquilla; 5 = Ciénaga; 6 = Dibulla; 7 = Riohacha.

DNA extraction

Fifty microliters grinding buffer (a mixture of sodium chloride, sucrose, Tris, ethylenediaminetetraacetic acid, and sodium dodecyl sulfate in pure water) was added to 1.5-mL microcentrifuge tubes in which adult mosquitoes had already been placed. After each sample had been individually homogenized, they were centrifuged for 2 min. The tubes were incubated at 65ºC for 30 min in a water bath. Seven microliters of 8 M potassium acetate was added to each tube; these were immediately removed from incubation while still hot. The tubes were placed on ice for 30 min; once this time had elapsed, they were centrifuged at 14,000 rpm for 15 min, and the supernatants were transferred to fresh microcentrifuge

tubes. One hundred microliters 100% cold ethanol was added to each of these tubes; after mixing the content in each one, they were incubated at room temperature for 5 min, and the ethanol was then carefully removed. One hundred microliters 70% cold ethanol was added to the precipitate in each tube, suspended, and centrifuged at 14,000 rpm for 5 min; the ethanol was carefully removed (in some cases, samples remained in ethanol overnight). One hundred microliters 100% cold ethanol was added to the pellet in each tube, and the tubes were centrifuged. The supernatant was carefully removed in such a way that the precipitate did not become detached. The alcohol was totally eliminated by inverting the tubes over paper towels and leaving them in this position for 2 h. One hundred microliters ultra-pure water was added to each tube; the DNA content was transferred, separately, to previously labeled storage vials, which were stored at -20°C until use (Coen et al., 1982).

RAPD-PCR and amplification conditions

Fourteen 10-bp primers (Invitrogen, USA) were used: A2, A09, A10, A20, B03, B3, B13, B16, B18, B20, C13, C19, E07, and E19, each having 10 bp. Nine polymorphic primers were selected from them according to their efficiency and reproducibility in PCR amplification. Table 1 shows the primers selected with their corresponding sequence. Amplification reproducibility was tested using the same samples in three different PCR reactions. One hundred twenty-two individuals (N = 122) corresponding to the 7 populations were distributed as follows: 15 from San Bernardo del Viento; 18 from each of Coveñas, Cartagena, Barranquilla, Ciénaga, and Riohacha (each one); and 17 from Dibulla. RAPD-PCR amplification was performed in a 25-µL mixture consisting of 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM dNTP, and 1 U *Taq* DNA polymerase with 10 ng DNA and 2 µM each primer.

The reaction was developed on an MJ-Research, Inc. thermocycler in the following conditions: initial denaturing at 95ºC for 5 min; 45 one-min cycles at 94, 55, and 72ºC; and a final extension step at 72ºC for 5 min. After being amplified, the samples were placed on 1.5% agarose gel and stained with ethidium bromide (0.5 µg/mL). The electrophoretic system was run for 1 h at a constant 150 V. Bands were visualized under ultraviolet light, and gels were photographed with a 667 Polaroid camera.

Genetic analysis

RAPD markers were analyzed using the following suppositions: a) RAPD alleles segregated in a Mendelian way; b) co-migrating bands are homologues; c) different loci are independently segregated; and d) populations are in Hardy-Weinberg equilibrium. Estimated allelic frequencies (calculated on the foregoing suppositions) were used to estimate the expected unbiased heterozygosity (Nei, 1978), the Shannon's diversity index, the genetic heterogeneity (G_{ST}) among the populations being considered (Nei, 1973), and the unbiased Nei genetic distance (Nei, 1978) among all of the population pairs. Gene flow (N_m) was calculated using the G_{ST} statistic and taking into account an n-dimensional island model (Takahata, 1983), where $N_m = (1 / G_{ST} - 1) / 4\alpha$, $\alpha = [n / (n - 1)] \times 2$, and n is the number of populations analyzed. Calculations were made using the statistical POPGENE genetic population package, version 1.32 (Yeh et al., 1997). For statistical analysis, the RAPD banding information was coded as a matrix of 1's (band present) and 0's (band absent).

Spatial autocorrelation

A spatial autocorrelation analysis was used to determine whether there was a significant spatial structure in the gene frequency patterns of variation among samples of the populations analyzed. We selected 24 of 75 polymorphic loci to analyze the possible spatial structure of the mosquito species being studied. These polymorphic loci are those that presented allele frequencies with higher values distant to 0. Spatial autocorrelation exists when the value of a variable in a population depends on the value of that same variable in the neighboring populations. On the other hand, when the value of a variable is independent, there is no spatial autocorrelation. The above method has been described by Sokal et al. (1989). This type of analysis involves 4 different steps. 1) A homogeneity test is required to establish the degree of genetic differentiation among populations of the mosquito studied on the Colombian Atlantic coast; for this purpose, we used the statistical probability corresponding to G_{ST}. 2) The autocorrelation coefficients and correlograms must be calculated. Moran's I index (Sokal and Oden, 1979) was used in this study. Additionally, 2 spatial autocorrelation analyses were performed: the first one used 3 classes of distances (CDs) (upper limits: 124, 220, and 413 km) and the other used 5 CDs (upper limits: 97, 154, 215, 261, and 413 km). The criterion used to select each specific class of geographic distance was chosen to optimize the location of the population pairs within each distance class. To determine the statistical significance of autocorrelation, the Bonferroni procedure was used (Oden, 1984). The percentage of significant autocorrelation coefficients was obtained to determine if it was superior to the 5% type I error. 3) Similarity analysis among the variable surfaces was performed. The similarity of gene surfaces was studied using the product-moment correlation coefficient of Pearson (r). The number of correlations superior to |0.7| was counted. Values greater than this count indicate markers that have similar surfaces. 4) The similarity among the correlograms must be determined. This was calculated by the Manhattan distance matrices between correlogram variable pairs generated with Moran's I index. This analysis was useful to determine if each one of the genetic variables studied was subjected to the same evolutionary processes. Sokal et al. (1989) showed, by means of simulation studies, that correlogram pairs generated by the same evolutionary processes have Manhattan distances lower than 0.1 in the case of Moran's I index. For this reason, the percentage of Manhattan distance values lower than these amounts was considered to be comparable to the 5% type I error.

RESULTS

Polymorphic RAPD markers

One hundred twenty-two specimens were evaluated based on 9 polymorphic RAPD markers (A2, A-09, A10, B-3, B-03, C-19, E-19, B-16, and B-20). Seventy-five of 119 (63.02%) amplified fragments were polymorphic. The size of these fragments ranged from 350 to 3600 bp, and there was an average of 8.3 amplified fragments per primer (Table 1).

RAPD-PCR genetic analysis of mosquito populations

The mean expected average heterozygosity for the mosquito populations studied was 0.358

(Table 2). Individually, most populations had similar values, with Barranquilla showing the highest value (0.371 ± 0.1289) and Riohacha being the population with the lowest value (0.343 ± 0.1163).

Table 1. Polymorphic primers, amplified fragment size, and maximum number of bands for each primer, corresponding to *Ochlerotatus taeniorhynchus* mosquito populations analyzed from the Colombian Atlantic coast.

Polymorphic primers	Nucleotide sequence	Size (bp)	Maximum number of bands
A2	5'-TGCCGAGCTG-3'	2800-550	10
A09	5'-GGGTAACGCC-3'	1900-350	6
A10	5'-ACGGCGTATG-3'	3600-1700	11
B3	5'-CATCCCCCTG-3'	2950-860	10
B03	5'-ACTTCGACAA-3'	2100-460	7
C19	5'-GGGTAACGCC-3'	3250-1500	7
E19	5'-ACGGCGTATG-3'	3180-930	8
B16	5'-TTTGCCCGGA-3'	2300-950	8
B20	5'-GGACCCTTAC-3'	3180-900	8

Table 2. Genetic variability statistics regarding each of the *Ochlerotatus taeniorhynchus* populations in the Colombian Atlantic coast.

Populations	Effective number of alleles	Expected heterozygosity	Shannon's diversity index
	N_E	H_E	I
San Bernardo	1.6353 ± 0.2790	0.368 ± 0.1211	0.5461 ± 0.1470
Coveñas	1.6378 ± 0.2768	0.369 ± 0.1211	0.5481 ± 0.1432
Cartagena	1.5983 ± 0.2755	0.354 ± 0.1211	0.5307 ± 0.1434
Barranquilla	1.6477 ± 0.2887	0.371 ± 0.1289	0.5475 ± 0.1589
Ciénaga	1.5843 ± 0.2784	0.348 ± 0.1216	0.5236 ± 0.1448
Dibulla	1.6004 ± 0.2728	0.355 ± 0.1173	0.5325 ± 0.1417
Riohacha	1.5665 ± 0.2557	0.343 ± 0.1163	0.5180 ± 0.1440

The overall expected heterozygosity for all of the populations taken together (H_T) was 0.39 ± 0.07. Meanwhile, the same value at the subpopulation level (H_S) was 0.37 ± 0.07.

The mean genetic heterogeneity among the populations analyzed yielded a value of G_{ST} = 0.05 ± 0.01, which was not significant (Table 3). The mean gene flow estimate among all the population pairs was 7.32 ± 1.35, which was very high. The Nei's genetic distance matrix (Table 4) showed that all of the values obtained were small. However, the values of the Riohacha population were slightly higher than the values from the other populations. The relationships among these 7 populations can be seen in Figure 2.

Table 3. Genetic structure and gene flow estimates among *Ochlerotatus taeniorhynchus* populations at the Colombian Atlantic coast.

Populations	H_T	H_S	G_{ST}	N_m
San Bernardo del Viento	0.250 ± 0,101	0.230 ± 0100	0.040 ± 0.03	9.62 ± 6.64
Coveñas	0.365 ± 0.059	0.329 ± 0.057	0.035 ± 0.02	7.27 ± 5.08
Cartagena	0.444 ± 0.036	0.422 ± 0.034	0.042 ± 0.02	7.09 ± 5.21
Barranquilla	0.448 ± 0.050	0.431 ± 0.048	0.037 ± 0.02	7.14 ± 4.58
Ciénega	0.458 ± 0.043	0.427 ± 0.042	0.068 ± 0.03	7.04 ± 2.33
Dibulla	0.400 ± 0.118	0.383 ± 0.112	0.041 ± 0.02	6.92 ± 2.88
Riohacha	0.364 ± 0.127	0.343 ± 0.112	0.056 ± 0.04	6.19 ± 4.30
Average	0.39 ± 0.07	0.37 ± 0.07	0.05 ± 0.01	7.32 ± 1.35

H_T = total gene diversity; H_S = gene diversity within subpopulation; G_{ST} = mean genetic heterogeneity of each population regard to all the other pairs; N_m = gene flow estimates of each population regard to all the other pairs.

Table 4. Nei's genetic distance matrix among the seven *Ochlerotatus taeniorhynchus* studied at the Colombian Atlantic coast.

	San Berna	Coveñas	Cartagena	Barranquilla	Cienaga	Dibulla	Riohacha
San Berna	0.0000						
Coveñas	0.0347	0.0000					
Cartagena	0.0374	0.0364	0.0000				
Barranquilla	0.0344	0.0271	0.0217	0.0000			
Cienaga	0.0358	0.0379	0.0242	0.0214	0.0000		
Dibulla	0.0419	0.0410	0.0237	0.0226	0.0232	0.0000	
Riohacha	0.0526	0.0518	0.0348	0.0289	0.0279	0.0292	0.0000

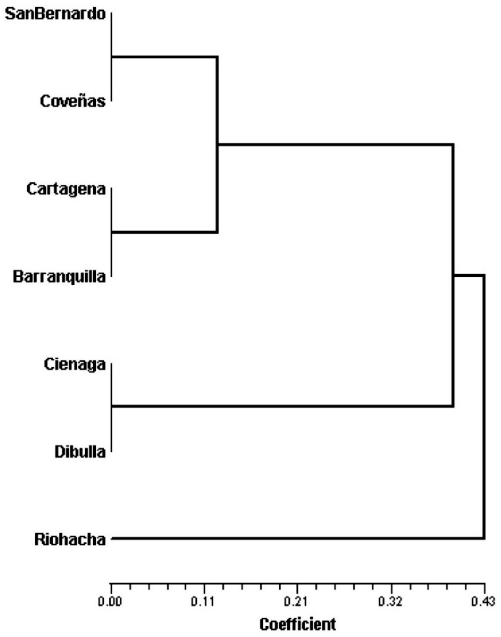

Figure 2. Dendrogram produced by the UPGMA algorithm with Nei's genetic distance (1978) applied to seven *Ochlerotatus taeniorhynchus* populations on the Colombian Atlantic coast.

Spatial autocorrelation

The spatial autocorrelation analysis of the 24 alleles selected for 3 CDs showed percentages of significant spatial autocorrelation coefficients that did not differ from the 5% type I error (Table 5A). Nine of 72 (12.5%) autocorrelation coefficients showed significant values. This value did not significantly differ from 5%. No alleles showed significant overall correlograms. Three alleles (B3-4, A09-1, and A09-2) showed correlograms that agreed with isolation by distance, but they were not significant. The spatial autocorrelation analysis for 5 CDs, using the same index, produced 10% significant spatial autocorrelation coefficients (12/120) (Table 5B); this percentage was not significantly different from the 5% error I margin (χ^2 = 2.16, d.f. = 1, P = 0.14). The alleles that showed significant overall correlograms for 5 CDs were A10-1, A10-2, B20-1, and C19-2. A10-1 and A10-2 showed correlograms that were simi-

lar to circular clines. Meanwhile, B20-1 and C19-2 showed correlograms that were related to isolation by distance or differentiation at a large distance. However, the number of significant overall correlograms was low. Therefore, both spatial autocorrelation analyses showed a weak spatial structure in *O. taeniorhynchus* from the Colombian Atlantic coast.

Table 5. Spatial autocorrelation analysis by using Moran's I index at 24 alleles selected from the populations of *Ochlerotatus taeniorhynchus* in the Colombian Atlantic coast.

A.

CD	124	220	413	PG
Alelos				
A10-1	0.12	-0.45	-0.16	0.405
A10-2	-0.46	-0.26	0.22	0.221
A10-3	-0.12	-0.12	-0.26	1.000
A10-4	**-0.58***	**-0.01**	**0.09**	**0.132**
A2-1	-0.43	0.00	-0.07	0.578
A2-2	-0.24	0.16	-0.45	0.283
A2-3	-0.48	-0.02	0.00	0.470
A2-4	**-0.54**	**-0.29**	**0.33***	**0.121**
B20-1	0.18	-0.11	-0.57	0.164
B20-2	0.10	-0.17	-0.43	0.170
B16-1	0.19	-0.18	-0.50	0.270
B16-2	**-0.38**	**0.31***	**-0.43**	**0.122**
E19-1	0.04	-0.48	-0.06	0.360
E19-2	0.05	-0.45	-0.10	0.422
C19-1	0.09	-0.05	-0.55	0.267
C19-2	0.19	-0.08	-0.61	0.183
B03-1	-0.37	0.18	-0.31	0.284
B03-2	0.26	-0.57	-0.19	0.228
B3-1	**0.38***	**-0.62**	**-0.26**	**0.127**
B3-2	-0.42	-0.00	-0.08	0.462
B3-3	**-0.77***	**0.34***	**-0.07**	**0.070**
B3-4	**0.39***	**-0.44**	**-0.45**	**0.111**
A09-1	**0.27**	**-0.15**	**-0.62***	**0.135**
A09-2	**0.26***	**-0.22**	**-0.54**	**0.146**
Average	-0.09	-0.15	-0.25	

B.

CD	97	154	215	261	413	PG
Alelos						
A10-1	**-0.08**	**0.29**	**-1.04****	**0.22**	**-0.20**	**0.044**
A10-2	**0.02**	**-1.13****	**-0.11**	**0.32**	**0.02**	**0.025**
A10-3	**-0.03**	**0.15**	**0.13**	**-0.01***	**-0.09**	**0.113**
A10-4	-0.66	-0.17	0.22	-0.29	0.02	0.404
A2-1	**-0.05**	**-0.77**	**-0.05**	**0.70***	**-0.56**	**0.097**
A2-2	-0.51	0.01	-0.13	-0.29	0.04	0.956
A2-3	**-0.94***	**0.24**	**-0.02**	**0.15**	**-0.24**	**0.233**
A2-4	**-0.21**	**-1.05***	**0.16**	**0.38**	**-0.12**	**0.062**
B20-1	**0.39**	**-0.06**	**-0.28**	**0.29**	**-0.98****	**0.027**
B20-2	**0.05**	**0.17**	**-0.38**	**0.06**	**-0.62***	**0.221**
B16-1	0.47	-0.03	0.07	-0.76	-0.50	0.257
B16-2	-0.50	-0.17	0.26	-0.19	-0.22	0.656
E19-1	-0.24	0.24	-0.49	-0.62	0.18	0.689
E19-2	-0.36	-0.04	-0.29	-0.21	0.02	1.000
C19-1	-0.35	0.27	0.29	-0.79	-0.23	0.408
C19-2	**0.45**	**-0.16**	**-0.08**	**0.19**	**-1.02****	**0.036**
B03-1	-0.48	-0.20	0.41	-0.18	0.34	0.302
B03-2	0.15	-0.27	-0.07	-0.65	-0.03	0.685
B3-1	0.27	0.19	-0.30	-0.83	-0.17	0.342
B3-2	**-0.00**	**-0.74***	**-0.01**	**0.33**	**-0.37**	**0.247**
B3-3	-0.44	-0.63	0.25	-0.23	0.13	0.571
B3-4	0.15	0.38	-0.16	-0.79	-0.37	0.386
A09-1	**0.30**	**0.18**	**0.28**	**-1.06***	**-0.46**	**0.084**
A09-2	**0.55***	**-0.13**	**-0.16**	**-0.31**	**-0.66**	**0.151**
Average	-0.09	-0.14	-0.06	-0.23	-0.28	

A. with three distance classes. *P < 0.05; **B.** with five distance classes. P < 0.05; **P < 0.01. Distances classes (CD) in kilometers. In bold alleles with some significant trend.

The percentage of allele correlations that exhibited values greater than |0.7| was 8.33% (23/276), which was not significantly higher than the type I error of 5% ($\chi^2 = 2.46$, d.f. = 1, P = 0.117). This indicated that there was a weak correlation between the frequency surfaces. On the other hand, the percentages of correlogram pairs with Manhattan distances lower than 0.1 were 9.05% (25/276) ($\chi^2 = 3.47$; d.f. = 1, P = 0.062) and 1.08% (3/276) for 3 and 5 CDs, respectively. Neither of these values was significantly higher than the type I error of 5%. Therefore, there were no global evolutionary forces that similarly affected the variables analyzed.

Additionally, we constructed unweighted pair group method with arithmetic mean (UPGMA) trees from Manhattan distance matrices among correlogram pairs for both 3 and 5 CDs. The results are shown in Figure 3A and B. The UPGMA analysis with 3 CDs showed some spatially relevant associations, including A2-2, BO3-1, and A2-2; A2-1, B3-2, and A2-3; A10-2 and A2-4; B20-2 and C19-1; B20-1, C19-2, A09-1, B16-1, and A09-2; B03-2 and B3-1; and A10-1, E19-1, and E19-2. With 5 CDs, the spatial associations that were identified included A2-1 and B3-2; A10-2 and A2-4; B20-1 and C19-2; A2-2 and E19-2; A10-4, B16-2, and B03-1; and B3-1 and B3-4. The fact that both analyses yielded some different allele associations agreed quite well with the idea that there were no clear evolutionary forces acting simultaneously upon these alleles in the same way.

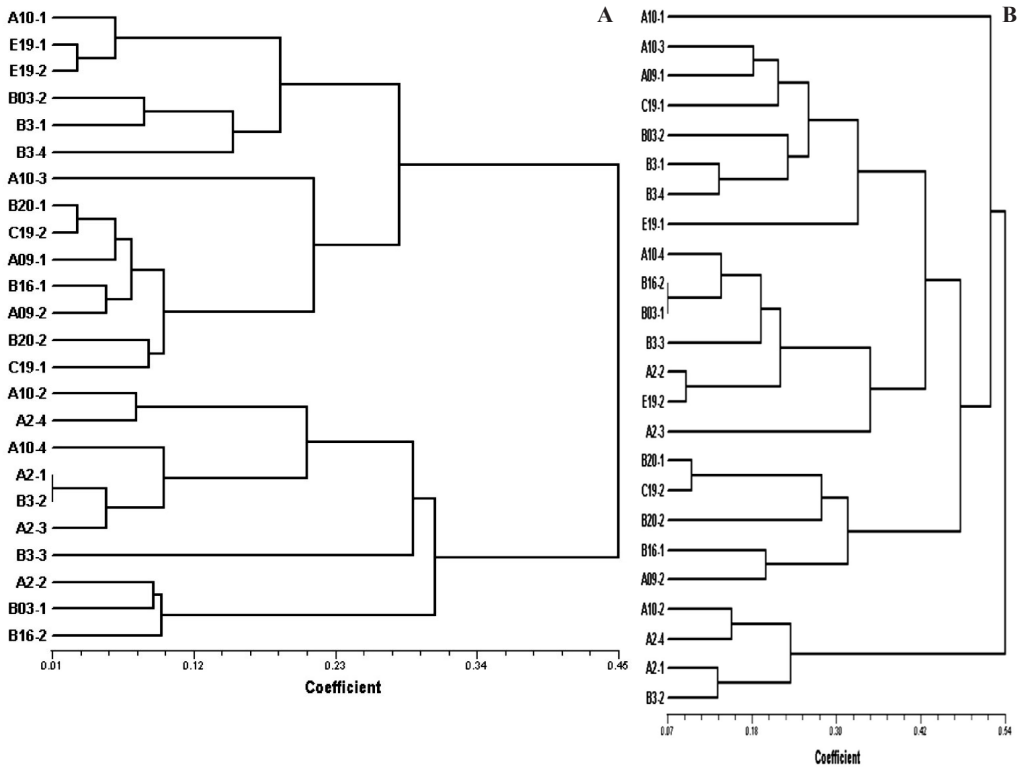

Figure 3. UPGMA trees, built from the Manhattan distance matrices obtained from correlograms with the Moran's I index among the 24 alleles selected in the seven *Ochlerotatus taeniorhynchus* populations studied at the Colombian Atlantic coast. **A.** Using three distance classes; **B.** using five distance classes.

DISCUSSION

The relatively high number of amplified fragments, as well as their high polymorphism percentages, reflected the consistent efficiency of the primers used in this study. This agrees with the results of other genetic analyses of populations corresponding to several vector insects (mainly mosquitoes), where their reproducibility and polymorphism have also been shown (de Sousa et al., 1999; Gorrochotegui-Escalante et al., 2000; Fonseca et al., 2001; dos Santos et al., 2003; Julio et al., 2009). For instance, Ayres et al. (2002), in study done on genetic diversity in 6 Brazilian *Aedes albopictus* populations, obtained just reproducibility and efficiency in 4 of the 10 primers tested. They obtained 47 amplification fragments having an average of 10 bands per primer. Even though the authors only took polymorphic primer records into account, our study showed higher levels of efficacy than their study. Likewise, Posso et al. (2003) obtained 65 polymorphic fragments from 10 primers in a study of the genetic variability and structure of 3 Colombian *Anopheles nuñeztovari* mosquito populations, which was an efficacy that was similar to that reported herein. More recently, Sharma et al. (2009), in a study on the genetic variability of 14 *Culex quinquefasciatus* populations from India, obtained a total of 81 polymorphic bands from 21 loci, reaching a level up to 100% polymorphism. Globally, these results showed relatively similar or slightly lower efficacy levels, but all of them, in turn, revealed a level of reproducibility and efficiency with RAPD primers that was similar to the level that we obtained.

Expected heterozygosity in *O. taeniorhynchus* populations, established with RAPDs, showed an average value of 0.358. Similar data were recorded in other studies with some mosquito species populations. For example, Gorrochotegui-Escalante et al. (2000) determined a value of 0.339 for *Aedes aegypti* populations along the northeastern coast of Mexico. The same species studied in populations from Puerto Rico had an average value of 0.354 (Apostol et al., 1996). Identically, Julio et al. (2009) estimated similar levels of genetic variability (0.351-0.404) in population samples of this mosquito from different neighborhoods in Córdoba City, Argentina. In addition, Posso et al. (2003) recorded an average value of 0.340 in the study of 3 Colombian *Anopheles nuñeztovari* populations. Results revealed similar heterozygosity (an average value of 0.365) in some *Aedes albopictus* populations in Brazil; this value is similar to the values obtained for other mosquito populations of this same species in the USA, the Malayan Peninsula, and Borneo (Black et al., 1988). Therefore, all of these species, including *O. taeniorhynchus*, demonstrated similar levels of genetic variability when this parameter was compared among the populations. On the contrary, lower average heterozygosities were established in other genetic analyses of mosquito populations. This is the case, for instance, in the study of Fonseca et al. (2001) in North American *Aedes japonicus* populations (0.09 to 0.19 heterozygosity).

The average genetic heterogeneity among the populations analyzed was $G_{ST} = 0.05 \pm 0.01$, which was indicative of scarce genetic differentiation among these *O. taeniorhynchus* populations on the Colombian Atlantic coast. This was probably determined by high gene flow levels ($N_m = 7.32$) among the populations of this species on the Colombian Atlantic coast. Among other Neotropical insect species, similar results of genetic heterogeneity have been observed with RAPDs. For example, Posso et al. (2003) estimated an $F_{ST} = 0.070$ among several *Anopheles nuñeztovari* mosquito populations in Colombia; this value is similar to that reported here.

It is interesting to compare these genetic heterogeneity and gene flow values that were obtained with RAPDs with those previously estimated with isoenzymes and microsatellites for the same populations. Bello and Ruiz-García (2009) estimated a genetic heterogeneity of F_{ST} = 0.069 among the same mosquito populations by means of isoenzymes, as well as a gene flow estimate of N_m = 3.39. Bello and Becerra (2009) estimated values of F_{ST} = 0.0369 and N_m = 4.97 using microsatellites in these same populations (all of the gene flow estimates were corrected for an n-dimensional island model). Thus, all of these genetic heterogeneity coefficients, as well as the gene flow estimates, were very similar independently of each one of the 3 types of markers employed, which represented completely neutral markers (RAPDs), neutral markers with some regulatory functions (microsatellites), and structural markers (isoenzymes). The highest genetic heterogeneity and the lowest value of gene flow that was obtained with isoenzymes could be related to a major pressure of differential natural selection in each locality studied, whereas RAPDs and microsatellites showed a more important neutral dynamic. Nevertheless, generally speaking, gene flow seems to be the most important evolutionary force, which modulates the current allele frequencies of the 3 kinds of markers that were studied in this mosquito species on the Colombian Atlantic coast. This disagrees with those observed in other organisms. For instance, Li et al. (2000), in 4 different wild emmer wheat populations, estimated that isoenzymes had a very elevated degree of heterogeneity (G_{ST} = 0.437), and microsatellites showed a lower degree of heterogeneity (G_{ST} = 0.291), while RAPDs yielded the lowest genetic heterogeneity (G_{ST} = 0.236). The same was observed for the Asian water buffalo (Barker et al., 1997), with isoenzymes showing a significantly higher genetic heterogeneity than microsatellites. In contrast and in agreement with our results, other organisms have shown similar levels of genetic heterogeneity in isoenzymes, RAPDs, and microsatellites. This was the case observed in *Typha latifolia* (Keane et al., 1999) and several wild cereals (Li et al., 1999).

The phenetic analysis with Nei's genetic distance and the UPGMA algorithm among the 7 *O. taeniorhynchus* populations showed differences that were extremely small (0.0214 to 0.0526), meaning that they cannot be considered genetically different populations. However, it is necessary to point out that there was a certain west-to-east spatial trend on the Colombian Atlantic coast, with the Riohacha population (extreme east) differing from the other 6 populations. The same was determined for isoenzymes (Bello and Ruiz-García, 2009). Our Nei's genetic distance values were notably lower than those obtained by Ayres et al. (2002). Ayres et al. (2002) analyzed different Brazilian *Aedes albopictus* populations, and the genetic distances that they obtained (0.050 to 0.133) were higher than those reported here. Thus, other species of mosquitoes in South America have shown less dispersion capacity than the species that we reported on the Colombian Atlantic coast.

The spatial autocorrelation analyses, with different classes of distances defined, clearly demonstrated that no significant spatial structure existed among the mosquito populations on the Colombian Atlantic coast. These results agree quite well with a model in which gene flow is high and random (Ruiz-Garcia, 2000). The same was discovered at isoenzyme markers for the same mosquito species by Bello and Ruiz-García (2009) when a Mantel test was applied. No significant spatial structure was detected. The fact that the degree of similarity among the variable surfaces and among the correlograms was low reflected that the genetic resemblance of the populations studied was not caused by a unique migratory movement. It also suggested that the genetic homogeneity was probably caused by a continuous and random

gene flow, with a restrictive random gene drift, along a major part of the Colombian Atlantic coast. In contrast, other studies, such as that of Foley et al. (2004), showed a clear significant spatial structure in mosquito species. They found a strong spatial structure in the peridomestic mosquito *O. notoscriptus*, with correlograms of different loci showing positive and significant values in the first distance classes and significant and negative values at the largest distances, reflecting isolation by distance.

Therefore, we can infer that this genetic resemblance of the mosquito populations that were analyzed could have a strong epidemiological impact because of the probable similar vector competence to transmit different VEEV immunological subtypes throughout this wide and endemic geographic region. Consequently, the design of control strategies of these mosquito populations should focus in the same way throughout this Colombian geographic area.

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