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Psorophora columbiae and *Psorophora toltecum* (Diptera: Culicidae) Colombian populations cannot be differentiated by isoenzymes

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ABSTRACT. Two populations of the mosquito *Psorophora columbiae* from the central Andean area of Colombia and one population of *Ps. toltecum* from the Atlantic coast of Colombia were analyzed for 11 isoenzyme markers. *Psorophora columbiae* and *Ps. toltecum* are two of the main vectors of Venezuelan equine encephalitis. We found no conspicuous genetic differences between the two species. The relatively high gene flow levels among these populations indicate that these are not two different species or that there has been recent divergence between these taxa. In addition, no global differential selection among the loci was detected, although the α-GDH locus showed significantly less genetic heterogeneity than the remaining loci, which could mean that homogenizing natural selection acts at this locus. No isolation by distance was detected among the populations, and a spatial population analysis showed opposite spatial trends among the 31 alleles analyzed. Multiregression analyses showed that both expected heterozygosity and the average number of alleles per locus were totally determined by three variables: altitude, temperature and size of the human population at the locality. Indi-

vidually, the expected heterozygosity is more related to these three variables than to the average number of alleles.

Key words: Population genetics, *Psorophora columbiae*, *Psorophora toltecum*, Isoenzymes, Genetic structure, Colombia

INTRODUCTION

Psorophora columbiae and *Ps. toltecum* are two mosquito species of medical and veterinary importance due to their efficacy as vectors of Venezuelan equine encephalitis (VEE) virus (PAHO, 1972). This is a potentially fatal disease that affects both humans and horses indigenous to northern South America. Both species have a wide geographical distribution, from the United States and Mexico to southern Colombia (Lanzaro, 1997). In Colombia *Ps. columbiae* seems to be present in the Andean areas, whereas *Ps. toltecum* is present in the Colombian Atlantic coast. In certain areas in Colombia both species could be sympatric. Another species, *Ps. confinnis*, has a geographical distribution from southern Colombia to Argentina.

Periodical human and equine epidemics of VEE have affected Colombia since 1938 (Dickerman et al., 1986). The last recorded epidemic occurred in the Colombian-Venezuelan State of Guajira in 1995. From 75,000 to 100,000 people were affected, with 3,000 neurological complications and 300 fatal cases. VEE has affected other American countries. In 1971, an outbreak of VEE in Texas caused 2,000 equine deaths with about 100 human infections. There were other VEE outbreaks in 1993 and 1996 in southern Mexico.

In areas where the two species are sympatric, hybrids are often found. In fact, there are hybrid zones in central Texas and in northern Mexico where hybridization occurs at relatively high frequencies at these sites (Lanzaro, 1997).

Members of species complexes could vary in vector capacity; this is critical to our understanding of disease epidemiology (Tabachnick and Black, 1995). Species complexes appear to be fairly common in many mosquitoes of medical importance (Wilkerson et al., 1995). Distinctive mtDNA lineages in the Amazon basin have been discovered for *Anopheles nuneztovari*, though they are not reflected in morphological traits of either males or females (Hribar, 1995). Nonetheless, in other cases, there are striking differences in morphological characters in male genitalia, pupal and egg stages correlated with mtDNA restriction sites in related species, such as *An. rangeli* and *An. trinkae* (Conn et al., 1997). There are highly significant differences for three restriction endonuclease among the Venezuelan and Trinidadian *An. aquasalis* populations and those from Brazil, while two Brazilian populations are also highly differentiated for these molecular markers. However, *An. aquasalis* populations were found to have identical ribosomal DNA restriction profiles (Conn et al., 1993). Additionally, studies made on morphological, cytological and electrophoretic characters (Faran, 1980; Moncada-Pérez and Conn, 1992) have concluded that *An. aquasalis* is a single species, a highly polymorphic species or a species complex (Conn et al., 1993). Four species of the *Aedes dorsalis* group have large genetic differences in at least 9 of 18 loci; these are diagnostic for each species pair, though morphological variation among these species is low (Gimnig and Eldridge, 1999).

There could be a similar situation for *Psorophora* populations in Colombia as this taxa is found on both the east and west coast of Central America and part of South America (3500 km), its behavior is variable, and VEE vector status also varies throughout its range.

Population genetic studies are of considerable importance for an understanding of the population dynamics of vectors and their potential dispersion capacity. Craig and Hickey (1965) made the first study on population genetics of vector insects, with *Aedes aegypti*. Genetic studies help to determine 1) taxonomic status among cryptic species and therefore their different vector function, 2) gene variability within and among species, which is related to the effective numbers of the species, and can be used to estimate the adaptive capacity to selective forces in vector mosquitoes, and 3) the gene flow levels among the populations, to examine how resistance genes are distributed among different populations. Several outstanding examples of these points are as follows. Bloem (1990) and Munstermann (1988) solved diverse taxonomic problems with biochemical methods in the *Aedes varipalpus* and *Ae. annulipes* groups, respectively. Gimnig and Eldridge (1999) estimated the levels of genetic variability in four species of the *Ae. dorsalis* group. Coosemans et al. (1998) determined differential genotypes in urban and rural *An. gambiae* and malaria transmission capacity in southwestern Burkina Faso, and Gimnig et al. (1999) determined temporal and spatial genetic variation within and among populations of *Culex tarsalis* in California. Lerdthusnee and Chareonviriyaphap (1999) demonstrated the existence of different isoenzyme patterns in *Ae. aegypti* populations collected from pre- and post-*Bacillus thuringiensis* treatment sites in Thailand, and the allele differentiation within *Ae. aegypti* populations in the French Polynesia according to human population density (Paupy et al., 2000). Byrne and Nichols (1999) demonstrated a differential gene flow capacity of *Culex pipiens* within and outside of London Underground tunnels. Conn et al. (1997) found a higher dispersal capacity in *An. trinkae* than in *An. rangeli* in Bolivia, Ecuador and Venezuela. We analyzed for the first time the genetic differentiation of two supposedly separate species in Colombia (*Ps. columbiae* and *Ps. toltecum*) by multilocus enzymatic electrophoresis to describe their genetic variability. The genetic and spatial structure of these *Ps. columbiae* and *Ps. toltecum* populations was analyzed in three different Colombian regions to examine genetic cline variation patterns (Watada et al., 1986, who studied *Drosophila simulans*). Additionally, the genotypes obtained from the Granada population (*Ps. columbiae*) were compared with those of a cell line derived from embryo tissues of this population, which was established two years earlier during a previous survey of the Granada population. This was done to determine the effects of gene drift and other factors on the genotypes.

MATERIAL AND METHODS

Mosquito populations analyzed

Adult *Ps. columbiae* were sampled in traps placed about 1 km apart, near rice fields, at Ambalema (Tolima Department; 04º 47´10´´N, 76º 46´00´´W), and Granada (Meta Department; 03º 32´19´´N, 73º 42´02´´W), in Colombia. The Ambalema and Granada populations are separated by the Eastern Andean mountains. Adult *Ps. toltecum* were sampled in traps in Lórica (Cordoba Department; 09º 13´54´´N, 75º 49´11´´W) at the Colombian Atlantic coast. After the mosquitoes were identified, they were kept at -70ºC until electrophoretic analysis. Thirty to 50 individuals were analyzed from each population for 11 isoenzyme loci.

Electrophoretic analyses

Isoenzymes were analyzed using cellulose acetate electrophoresis (Titan III, Helena Laboratories), following the procedure described by Kreutzer and Christensen (1980). Each mosquito was homogenized in 15 µl H_2O. Isoenzyme loci analyzed were: malate dehydrogenase (MDH) (E.C. 1.1.1.37), phosphoglucomutase (PGM) (E.C. 5.4.2.2), isocitrate dehydrogenase (ICDH) (E.C. 1.1.1.42), leucine aminopeptidase (LAP) (E.C. 3.4.11.1), α-glycerophosphate dehydrogenase (α-GDH) (E.C. 1.1.1.8), malic enzyme (ME) (E.C. 1.1.1.40), hexokinase (HK) (E. C. 2.7.1.1), phosphoglucose isomerase (PGI) (E.C. 5.3.1.9), mannose-6-phosphate isomerase (MPI) (E.C. 5.3.1.8), 6-phosphogluconate dehydrogenase (6PGDH) (E.C. 1.1.1.44), and aspartate transaminase (ASAT) (E.C. 2.6.1.1).

Population genetic analyses

Allele frequencies and standard deviations were calculated for all polymorphic loci (Hedrick, 1985). The genetic variability for each of the populations was estimated by determining the number of alleles per locus, the proportion of polymorphic loci and the expected heterozygosity (Nei, 1973).

Hardy-Weinberg equilibrium (HWE) was measured by using Robertson and Hill's f statistic (1984). The significance was determined with a chi-square test. The hierarchical genetic structure of the populations surveyed was estimated with the F-statistic (Wright, 1951), by using the FSTAT program (Goudet, 1995). The significance of these F-statistics was measured with 500 bootstrap permutations of alleles and genotypes for each sample.

Indirect gene flow estimates were obtained from the F_{ST} statistic, where Nm is the product of the effective population size and the migration rate per generation, assuming an infinite island model (Wright, 1965), where $Nm = [(1/F_{ST}) - 1]/4$. The private allele model of Slatkin (1985) and Barton and Slatkin (1986) was also employed to obtain gene flow estimates. Estimates of F_{ST} and Nm were calculated for each population pair.

Three genetic distances with different mathematical properties were used to determine the relationships among the populations studied: Nei's (1978), chord Cavalli-Sforza and Edwards' (1967), and Rogers' (1972) genetic distances. UPGMA and neighbor-joining (Saitou and Nei, 1987) algorithms were employed to determine the degree of relationship among the three populations. In addition, the relationships among the alleles detected were analyzed by obtaining the correlation and the Euclidean distance matrices among these alleles from the polymorphic loci. The UPGMA and the neighbor-joining algorithms were applied to these matrices, as well as the strict and the Stinebrickner (s = 0.8) consensus trees, to determine the genetic relationships among the diverse alleles within the populations.

A multidimensional scaling analysis was carried out by the Kruskal (1964) procedure to generate a phenetic relationship among the three mosquito populations. Fifty iterations were used to calculate the "final stress" statistic, which measured the goodness of fit of the distances in the configuration space to the monotone function of the original distances. The 50 iterations were stopped if a "minimum stress" of less than 0.001, or a ratio of "maximum stress" higher than 0.999 were obtained. The relationships among the alleles found were analyzed with the same procedure to explore how the various alleles help to discriminate among the populations. The Lewontin-Krakauer test (1973) was used to determine whether selection has an important

effect on the genetic structure of the *Psorophora* populations studied. The theoretical basis of this test depends on the inexistence of significant heterogeneity among all the F_{ST} values analyzed for each marker, to determine if this variation is due to stochastic processes affecting the same parameters and/or interpopulational gene flow (which could be irrelevant if the taxa belong to different species), since these events would have to affect all the loci equally (Wood, 1978). On the contrary, the finding of significant heterogeneity would suggest the presence of selective agents differentially affecting the analyzed loci. A k = 2 was used since this preserves a magnitude that is appropriate for a binomial distribution and for F_{ST} values between 0 and 0.05 (Nevo, 1973). Furthermore, the Fisher-Snedecor test was applied to determine if the genetic heterogeneity was differential for all the possible marker pair comparisons. This could help to detect natural selection, or differential demographic processes, for individual markers that could be underdetermined with the Lewontin-Krakauer test, which analyzes all loci globally (Ruiz-Garcia and Alvarez, 2000).

Two procedures were employed to determine a possible spatial structure among the *Psorophora* populations: 1) the isolation-by-distance model created by Slatkin (1993). This method is based on the regression calculations between the estimated gene flow matrices among locality pairs from the F_{ST} and G_{ST} statistics, corrected for sample sizes and geographic distances among these localities. The expression used is $Log_{10}(Nm) = a + b \log_{10}$ (geographic distance). If the b value is high and significantly negative, it is concluded that there is strong isolation by distance. If not, isolation by distance is not accepted as a parsimonious explanation. To measure the significance of b, the Mantel test using a Monte Carlo simulation (2000 random permutations) was used as well as the Spearman rank-correlation coefficient applied to the F_{ST} matrix among population pairs and to the geographic distance matrix. The geographic distances between the populations was calculated by using the Spuhler (1972)'s distance, with the expression:

$D = \arccos(\cos X(I) \cdot \cos X(j) + \sin X(I) \cdot \sin X(j) \cdot \cos |Y(I) - Y(j)|)$, where X(n) and Y(n) are the latitude and longitude of the *nth* populations, respectively.

2) A spatial autocorrelation analysis was also applied by Moran's I index (Moran, 1950). Only two distance classes (DC) were defined due to the small number of populations. To determine the statistic significance of the autocorrelation coefficients, the Oden (1984) procedure was used. In addition, a single autocorrelation coefficient was obtained for each genetic variable. In this case, the point pairs were weighted as the inverse square of the separation distance between the localities. The percentage of significant autocorrelation coefficients was obtained to determine if it was superior to the 5% error margin. A similarity analysis of the correlograms was also undertaken. An average Manhattan distance matrix (Sneath and Sokal, 1973) between the autocorrelation coefficients estimated for variable pairs of correlograms was calculated to determine the similarity between the correlograms. This analysis can determine if each of the genetic variables studied was subjected to the same spatial evolutionary event, or if they were under pressure from different spatial evolutionary agents. Several of the major goals in population genetics obtained through the spatial autocorrelation analysis can be found in Sokal and Oden (1978a,b), Sokal et al. (1986, 1987, 1989), Ruiz-Garcia (1994a,b, 1997, 2000) and Ruiz-Garcia and Jordana (1997, 2000).

Lastly, multiple regression equations between expected heterozygosity and the average number of alleles per locus, and the temperatures, altitudes and the size of the human population of each locality were obtained. Natural selection can be detected with these proce-

dures. In this multiple regression analysis, the regression hyperplanes of the expected heterozygosity and the average number of alleles per locus (x_i) versus temperature (x_1), altitude (x_2), and human population numbers (x_3) were obtained using the minimum square method ($x_i - u_m = b_1 (x_1 - u_1) + b_2 (x_2 - u_2) + b_3 (x_3 - u_3)$, where u_i is the expected value of each variable (i = 1, 2…m) and b_i (i = 1, 2…m) is the regression coefficient of x_m on x_1, x_2, x_3). Furthermore, the square multiple correlation was calculated, which represents the goodness of fit between x_i, the observed value of each genetic variable, and the better linear predictor, x_i, which is a function of x_1, x_2, and x_3, and with the expression $P^2 = \Sigma_I (x_i - x_i)^2 / \Sigma_I (x_i - X)^2$, where X is the average of the values of the genetic variable taken as dependent. If P is near 0, the genetic variable will be highly uncorrelated with geographic and environmental variables. On the other hand, P = 1 indicates that x_i is an exactly linear combination of the four variables. The residual variances were calculated as well; these are the mean square distances of the points to the regression hyperplanes, R_o^2/n, with $R_o^2 = \Sigma_I (x_i - x_i)^2$, where x_i is the prediction of x_i as a function of the regression hyperplane and n the number of points compared, or, $var(x_i) = \sigma_i^2 (1 - P^2)$, where σ_i^2 is the variance of the observed distribution of each one of the genetic markers and P^2 is the square multiple correlation coefficient. The square root of this value is the typical error of the estimated x_i value. In addition, in order to observe whether the type of data has some effect on the correlations, linear, logarithmic, exponential and power functions were calculated among the variables.

RESULTS

Levels of genetic variability

Only two of 11 loci were monomorphic in the three populations; these were 6-PGDH and ASAT. Most of the polymorphic loci did not yield more than three alleles, with the exception of MPI, ICDH (four alleles) and MDH (five alleles). The frequency of the most common allele was not higher than 0.85, with the exception of α-GDH (0.97), and not lower than 0.40. Several alleles were found only in the two populations of *Ps. columbiae* (α-GDH-C, MDH-D and ME-B) and others only in the *Ps. toltecum* population (PGI-C and LAP-C). Therefore, these alleles seem to be diagnostic for these populations. The loci that yielded the highest number of alleles were MDH, ICDH and MPI; these were the loci with the highest levels of expected heterozygosity (H). In the case of MDH, H ranged from 0.530 (Granada) to 0.666 (Lorica), whereas, for MPI, H ranged from 0.604 (Ambalema) to 0.684 (Lorica). The isoenzymes that had the lowest levels of expected heterozygosity, were α-GDH (Lorica H = 0.042 and Granada H = 0.159) and ME (Ambalema H = 0.188, and Granada H = 0.315) (Table 1).

Average genetic variability was determined for each of the populations (Table 2). The average number of alleles per locus in each population ranged from 2.5 and 2.8. Polymorphic loci percentages were 81.8%, being the same for the three populations. Expected heterozygosity was also very similar among populations, ranging from 0.39 (Lorica) to 0.42 (Ambalema), with no significant differences.

Hardy-Weinberg equilibrium

Based on the Robertson and Hill (1984) f statistic, the deviation percentages from

Table 1. Allele frequencies and standard deviations of nine polymorphic isoenzymes of *Psorophora columbiae* populations in Ambalema and Granada and for *Psorophora toltecum* in Lorica, Colombia. n = number of specimens analyzed for each locus.

Locus	Ambalema (N = 50)	Granada (N = 43)	Lorica (N = 49)
PGM			
A	0.547 ± 0.055	0.484 ± 0.057	0.844 ± 0.041
B	0.453 ± 0.055	0.453 ± 0.056	0.141 ± 0.039
C	0.000 ± 0.000	0.063 ± 0.027	0.016 ± 0.014
HK			
A	0.854 ± 0.039	0.458 ± 0.056	0.833 ± 0.041
B	0.146 ± 0.039	0.167 ± 0.042	0.125 ± 0.039
C	0.000 ± 0.000	0.375 ± 0.055	0.042 ± 0.023
PGI			
A	0.667 ± 0.052	0.979 ± 0.016	0.708 ± 0.051
B	0.333 ± 0.052	0.021 ± 0.016	0.167 ± 0.042
C	0.000 ± 0.000	0.000 ± 0.000	0.125 ± 0.037
α-GDH			
A	0.083 ± 0.030	0.063 ± 0.027	0.021 ± 0.016
B	0.917 ± 0.030	0.917 ± 0.031	0.979 ± 0.016
C	0.000 ± 0.000	0.021 ± 0.016	0.000 ± 0.000
LAP			
A	0.341 ± 0.052	0.333 ± 0.053	0.481 ± 0.056
B	0.659 ± 0.052	0.667 ± 0.053	0.481 ± 0.056
C	0.000 ± 0.000	0.000 ± 0.000	0.038 ± 0.022
MPI			
A	0.139 ± 0.038	0.361 ± 0.054	0.361 ± 0.054
B	0.611 ± 0.054	0.472 ± 0.056	0.403 ± 0.055
C	0.194 ± 0.044	0.153 ± 0.041	0.167 ± 0.042
D	0.056 ± 0.025	0.014 ± 0.013	0.069 ± 0.029
MDH			
A	0.125 ± 0.037	0.135 ± 0.039	0.118 ± 0.036
B	0.306 ± 0.051	0.135 ± 0.039	0.408 ± 0.055
C	0.528 ± 0.055	0.662 ± 0.054	0.359 ± 0.055
D	0.028 ± 0.018	0.041 ± 0.022	0.079 ± 0.042
ICDH			
A	0.614 ± 0.054	0.806 ± 0.045	0.792 ± 0.046
B	0.043 ± 0.022	0.014 ± 0.013	0.028 ± 0.019
C	0.300 ± 0.051	0.097 ± 0.033	0.139 ± 0.039
D	0.043 ± 0.022	0.083 ± 0.031	0.042 ± 0.023
ME			
A	0.067 ± 0.028	0.034 ± 0.020	0.117 ± 0.037
B	0.033 ± 0.019	0.155 ± 0.041	0.000 ± 0.000
C	0.900 ± 0.033	0.810 ± 0.044	0.883 ± 0.036

α-GDH = α-glycerophosphate dehydrogenase; HK = hexokinase; ICDH = isocitrate dehydrogenase; LAP = leucine aminopeptidase; MDH = malate dehydrogenase; ME = malic enzyme; MPI = mannose-6-phosphate isomerase; PGI = phosphoglucose isomerase; PGM = phosphoglucomutase.

Hardy-Weinberg equilibrium (HWE) for the populations of Granada and Lorica were 22.2 and 33.3%, respectively. For Granada, the loci that were in HW disagreement were MDH (f = 0.3625, χ^2 = 19.453, 1 d.f., P = 0.0000) and MPI (f = 0.2208, χ^2 = 5.226, 1 d.f., P = 0.0217),

Table 2. Average number of alleles per locus, percentage of polymorphic loci and average expected heterozygosity for *Psorophora columbiae* populations (Ambalema, Granada) and for the *Psorophora toltecum* population (Lorica) in Colombia.

Population	Sample size	# alleles/locus	% Polymorphic loci	Expected heterozygosity
Ambalema	40	2.5	81.8	0.4217
Granada	42	2.8	81.8	0.4074
Lorica	45	2.7	81.8	0.3928

both to excess homozygotes. MDH disequilibrium was also apparent when the Bonferroni's procedure correction was applied. At Lorica, the loci that deviated from HWE were LAP (f = 0.4280, χ^2 = 9.526, 1 d.f., P = 0.0020), PGI (f = 0.8857, χ^2 = 37.655, 1 d.f., P = 0.0000) and HK (f = 0.5071, χ^2 = 12.342, 1 d.f., P = 0.0004), all them by homozygous excess. The disagreement for PGI and HK continued, even after applying Bonferroni's procedure correction. Ambalema was the population that yielded the highest degree of HWE deviations (7/9). These were the cases for MDH (f = 0.207, χ^2 = 6.557, P = 0.0104), α-GDH (f = 1.0000, χ^2 = 26.132, P = 0.0000), ME (f = 0.4882, χ^2 = 14.3, P = 0.0002), HK (f = 0.8726, χ^2 = 18.274, P = 0.0000) and MPI (f = 0.5778, χ^2 = 36.053, P = 0.0000), all of them by homozygous excess, while PGM (f = -0.8258, χ^2 = 21.823, P = 0.0000) and PGI (f = -0.4891, χ^2 = 5.742, 1 d.f., P = 0.0166) deviated by heterozygous excess. Nevertheless, when Bonferroni's correction was applied and several alleles with low frequencies joined, only the loci α-GDH, HK, MPI and PGM showed HW disequilibrium, which was significantly more than detected in Granada and Lorica.

Ambalema was the population that yielded the most highly biased HWE estimates. Markers that were not in HWE were different among the populations: PGM and PGI showed a significant heterozygous excess in Ambalema, not found in Lorica and in Granada. α-GDH yielded a significant homozygous excess in Ambalema, but not in Granada and Lorica. On the other hand, PGI and HK showed a significant skew from HWE in Ambalema and Lorica, but not in Granada. PGI yielded a heterozygous excess in Ambalema and was in HWE in Granada, whereas there were excess homozygotes in Lorica. Only Ambalema had two cases of heterozygous excess. ICDH was in HWE for the three populations.

Hierarchical population structure

Wright's F-statistics (F_{IS}, F_{ST}, and F_{IT}) were determined for nine polymorphic markers (Table 3). The significance levels were measured as follows: 500 bootstrap allele permutations within each subpopulation sample were made out to obtain F_{IS} significance with 95% intervals; 500 allele bootstrap permutations were carried out in the total sample to determine the levels of significance of F_{IT} and F_{ST} and another 500 genotype bootstrap permutations were obtained within the total sample to determine the F_{ST} significance level. The only locus for which there were no significant F-statistics was LAP.

The genetic markers that yielded a significant deviation at the subpopulation level (F_{IS}) were α-GDH, MPI, HK, all of them by homozygous excess, and PGM with a striking heterozygous excess as well as ICDH, with slightly significant heterozygous excess. Other markers

Table 3. Hierarchical F-statistics applied to the *Psorophora* populations for each of the nine polymorphic loci. Bootstrap for 99% confidence intervals and their respective probabilities for F_{IS}, on one hand, and for F_{IT} and for F_{ST}, on the other hand, by using 1000 permutations.

	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}	F_{IS}
MDH	-0.033	0.049	-0.087			
		99% confidence interval			99% confidence interval	
	-0.104	0.046	-0.162	-0.165	-0.013	-0.172
	0.193	0.050	0.154	0.160	0.039	0.156
P			0.87400	0.6800	0.00200*	
PGM	-0.388	0.133	-0.600			
		99% confidence interval			99% confidence interval	
	-0.082	0.120	-0.240	-0.260	-0.017	-0.254
	0.347	0.128	0.257	0.214	0.064	0.221
P			0.99800	0.99800	0.00200*	
ICDH	-0.094	0.043	-0.143			
		99% confidence interval			99% confidence interval	
	-0.138	0.039	-0.190	-0.156	-0.014	-0.172
	0.234	0.044	0.204	0.203	0.041	0.202
P			0.9680	0.87200	0.00200*	
α-GDH	0.415	-0.009	0.421			
		99% confidence interval			99% confidence interval	
	-0.052	-0.009	-0.053	-0.059	-0.023	-0.116
	0.415	0.001	0.421	0.413	0.078	0.421
P			0.00800*	0.00200*	0.57600	
ME	-0.005	0.027	-0.033			
		99% confidence interval			99% confidence interval	
	-0.097	0.022	-0.129	-0.112	-0.019	-0.136
	0.314	0.029	0.299	0.215	0.054	0.226
P			0.48200	0.42400	0.04200*	
HK	0.391	0.171	0.265			
		99% confidence interval			99% confidence interval	
	-0.045	0.170	-0.275	-0.208	-0.023	-0.201
	0.420	0.180	0.300	0.260	0.063	0.243
P			0.00600*	0.00200*	0.00200*	
LAP	-0.149	0.021	-0.173			
		99% confidence interval			99% confidence interval	
	-0.227	0.015	-0.254	-0.220	-0.016	-0.220
	0.241	0.022	0.229	0.254	0.077	0.260
P			0.93800	0.94800	0.0940	
PGI	0.290	0.136	0.179			
		99% confidence interval			99% confidence interval	
	-0.083	0.132	-0.266	-0.215	-0.022	-0.226
	0.477	0.144	0.398	0.262	0.106	0.250
P			0.06800	0.00200*	0.00200*	
MPI	0.388	0.026	0.371			
		99% confidence interval			99% confidence interval	
	-0.125	0.028	-0.163	-0.180	-0.014	-0.182
	0.203	0.0033	0.179	0.178	0.040	0.185
P			0.00200*	0.00200*	0.01800*	

For abbreviations, see legend to Table 1. * Significant probabilities of the respective F-statistics ($P < 0.05$).

gave negative, though nonsignificant, F_{IS} values (MDH, ME and LAP). Nevertheless, the overall average F_{IS} for the nine markers was low (F_{IS} = 0.022) for all markers taken together; meaning that globally there was no important deviation from HWE at the subpopulation level.

The F_{ST} statistic measures the genetic heterogeneity degree among all the subpopulations. All the markers employed, except for α-GDH and LAP, yielded significant heterogeneity. Those that showed the strongest heterogeneity were HK (F_{ST} = 0.171, range for α = 0.05, -0.020-0.038, P = 0.002), PGI (F_{ST} = 0.136, range for α = 0.05, -0.020-0.054, P = 0.002), and PGM (F_{ST} = 0.133, range for α = 0.05, -0.008-0.017, P = 0.002). Overall, the genetic differentiation among the three *Psorophora* populations was moderate (F_{ST} = 0.067).

The MDH, ICDH, ME and LAP markers gave no striking intra- or intersubpopulational differentiation (F_{IT}). On the other hand, the following markers were strongly significant: PGM, PGI, MPI, HK and α-GDH. These five markers gave negative F_{IT} values, although only one was significant (PGM), which means that there were more heterozygotes than those expected in HWE. Four markers yielded positive F_{IT} (excess homozygotes), all of them being significant (α-GDH, HK, PGI and MPI). The average value of F_{IT} was 0.090.

If we analyze the F_{ST} values among population pairs for each of the polymorphic markers (Table 4), it can be seen that each marker gave a heterogeneous relationship among the different variables.

Based on the average F_{ST} statistic, an estimate of theoretical gene flow (Nm) among the three populations was Nm = 3.48, which is substantially higher than 1, the value expected to equilibrate gene flow and gene drift in an infinite island model. In addition, the gene flow estimates using the private allele model gave Nm = 2.005, when all three populations were considered, and Nm = 2.17, when only two species were considered (*Ps. columbiae* versus *Ps. toltecum*), which is also higher than 1. Gene flow pair population estimates are shown in Table 4. There was a considerable variation in the values for each variable for each population pair. For instance, we found 9.258 (MDH), 84.687 (PGM), 3.094 (ICDH), infinite (α-GDH), 8.324 (ME), 0.804 (HK), infinite (LAP), 0.647 (PGI) and 6.364 (MPI) in the comparison of Ambalema and Granada.

Phylogenetic relationships among the populations

Different dendrograms were obtained by using different algorithms (UPGMA and neighbor-joining) and different genetic distances (Nei, Cavalli-Sforza and Edwards, and Rogers) (Figure 1). All the trees that were obtained were identical, independent of the algorithms and genetic distances used. The two populations of the central area of Colombia (Ambalema and Granada), *a priori* classified as *Ps. columbiae*, were found to be highly unrelated, whereas the Lorica population in the Atlantic Coast of Colombia, *a priori* classified as *Ps. toltecum*, was quite similar to the central population of Ambalema. The Nei's genetic distances (Table 5) for Ambalema and Granada and for Ambalema and Lorica indicated a moderate to strong genetic divergence among these populations, whereas among the two populations most geographically distant (Ambalema and Lorica), the genetic distance was lowest, which indicated a striking genetic similarity between the Atlantic population and one of the central Andean area populations (Ambalema). A multidimensional scale analysis, with Nei's distance, confirmed this fact, as well. The first axis clearly discriminated Ambalema and Lorica from Granada, and, in turn, explained 84% of the overall variance.

Table 4. F_{ST} and Nm (gene flow) pairwise values among three *Psorophora* populations from Colombia for nine polymorphic loci. Values above the main diagonal matrix are from the Nm statistic and values below the main diagonal matrix are from the F_{ST} statistic.

Enzyme	Populations		
	P. confinis (Ambalema) (1)	*P. confinis* (Granada) (2)	*P. toltecum* (Lorica) (3)
MDH			
1	-	9.258	15.985
2	0.0263	-	2.240
3	0.0154	0.1004	-
PGM			
1	-	84.687	1.061
2	0.0029	-	0.936
3	0.1906	0.2109	-
ICDH			
1	-	3.094	4.694
2	0.0748	-	infinite
3	0.0506	0.0000	-
α-GDH			
1	-	infinite	166.500
2	0.0000	-	24.109
3	0.0015	0.0103	-
ME			
1	-	8.324	infinite
2	0.0292	-	4.642
3	0.0000	0.0511	-
HK			
1	-	0.804	infinite
2	0.2372	-	1.003
3	0.0000	0.1995	-
LAP			
1	-	infinite	6.268
2	0.0000	-	5.443
3	0.0384	0.0439	-
PGI			
1	-	0.647	10.318
2	0.2786	-	1.407
3	0.0237	0.1509	-
MPI			
1	-	6.364	4.478
2	0.0378	-	infinite
3	0.0529	0.0000	-

For abbreviations, see legend to Table 1.

Genetic relationships among the alleles

The correlation and Euclidean distance matrices among each of the allele pairs of the different loci were obtained. Two main groups of variables were discriminated with the UPGMA

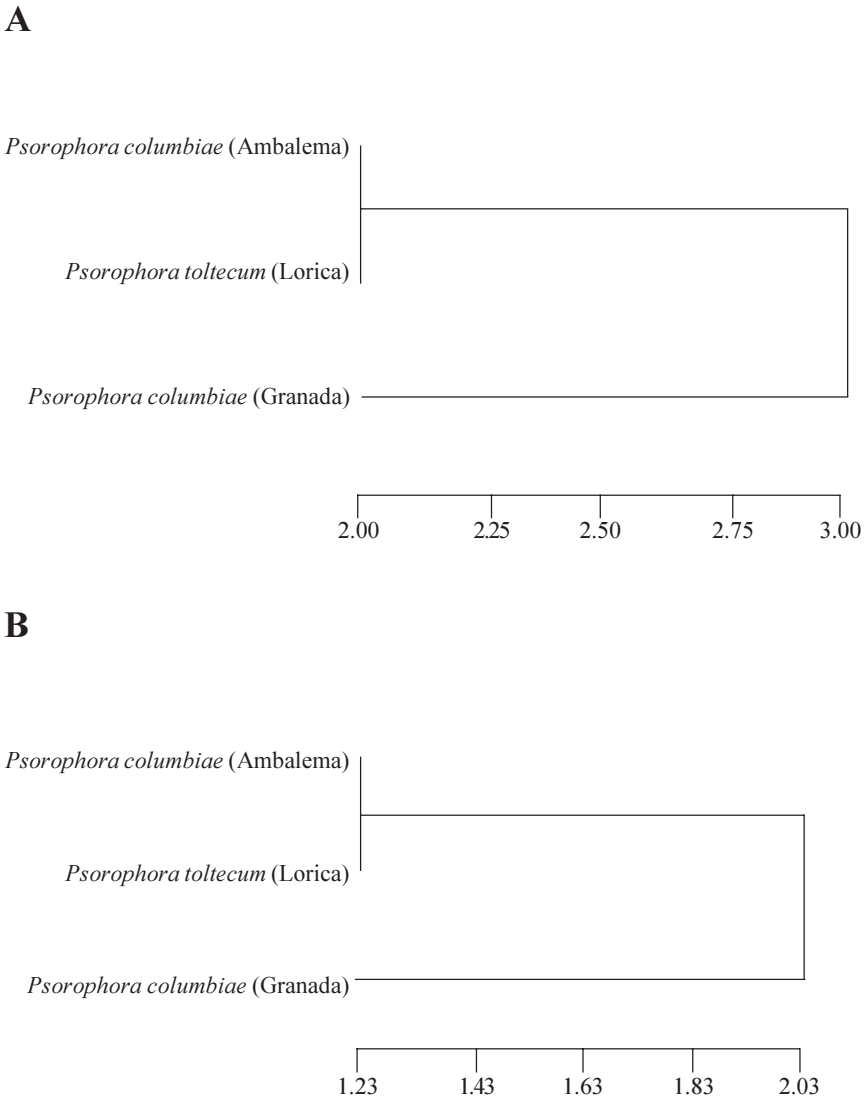

Figure 1. UPGMA trees for three *Psorophora* populations in Colombia. A, Nei's genetic distance; B, Cavalli-Sforza and Edwards' (1967) genetic distance.

Table 5. Genetic distance matrices among three *Psorophora* populations from Colombia. Values above the main diagonal matrix are Cavalli-Sforza and Edwards' (1967) genetic distance and values below the main diagonal matrix are Nei's (1978) genetic distance.

Population	*Psorophora columbiae* - Ambalema	*Psorophora columbiae* - Granada	*Psorophora toltecum* - Lorica
Psorophora columbiae - Ambalema	-	1.9554	1.2264
Psorophora columbiae - Granada	0.0705	-	2.1061
Psorophora toltecum - Lorica	0.0438	0.0814	-

algorithm and the correlation coefficient (Figure 2). Two other subclusters were found inside of each of these two major clusters. We found related alleles of the loci PGM, ME, PGI, α-GDH, LAP, MDH, MPI and ICDH, and of the loci HK, ME, MPI, MDH, PGI and ICDH, respectively, in subclusters 1 and 2 of the main cluster. In the second main cluster, alleles from PGM, MDH, LAP, α-GDH and MPI were related in subcluster 1, whereas in the subcluster 2, alleles from PGM, HK, α-GDH, ICDH, MDH and ME were found. Two main clusters were also determined with the UPGMA algorithm and the Euclidean distance, although the internal relationships among the variables were different from that found in the previous analysis. In the first cluster, the more strongly related alleles were PGM-A with HK-A, PGI-A with ICDH-A, α-GDH-B with ME-C and the association between PGM-B, LAP-B, MDH-C and MPI-B. The second main cluster was less structured than that found in the previous analysis. Several consensus trees among the different allele trees (Figure 3) revealed two main groups of allele relationships. The alleles whose relationships were most distant were LAP-A and B, MPI-A and B and MDH-B and C. A multidimensional scaling analysis with the Euclidean distances for the 31 alleles was made (Figure 4). This analysis clearly indicated that the alleles least related to the others with the first axis were LAP-B, ME-C, MDH-C, ICDH-A, α-GDH-B and PGI-A, while the alleles most differentiated in the second axis were PGM-B and HK-C. Therefore, these alleles gave the highest degree of discrimination in these populations.

Differential genetic heterogeneity among the genetic markers

The Lewontin and Krakauer (1973) test did not reveal any significant genetic heterogeneity when all the polymorphic loci were considered simultaneously, with k = 2 (S^2_{FST}/σ^2_{FST} = 0.847 with F (8, infinite)). Likewise, with a less constrictive value of k (= 1), this statistic did not detect any trace of differential heterogeneity among all the loci employed (S^2_{FST}/σ^2_{FST} = 1.694 with F (8, infinite)). Therefore, there is no evidence of differential selection affecting the loci. However, when we analyzed for the possible existence of significant differential heterogeneity among locus pairs with the F Fisher-Snedecor test, several significant cases were detected (Table 6). The α-GDH locus gave significantly less genetic heterogeneity than the remaining loci. Thus, this locus could be affected by homogenizing selection.

Relationships between the genetic and geographic distances of the mosquito populations

The relationships between the genetic and the geographic distances of these populations were analyzed. When the isolation by distance test (Slatkin, 1993) was applied to our data, no evidence was detected. The equation for the F_{ST} pair values and the Sphuler geographic distances was: $\text{Log}_{10}(F_{ST})$ = -0.47778422 – 0.77339812 Log_{10}(Sphuler geographic distance) with r = -0.993236, which means that the more distant populations were more related genetically. This first test, therefore, did not show evidence of isolation by distance.

Two spatial autocorrelation analyses made with Moran's I index were carried out. One of them had two DC, of different sizes (1 DC: 0-505 km; 2 DC: 505-667 km). The other analysis used two equal DC (1 DC: 0-338 km; 2 DC = 338-667 km). Despite no overall correlogram was significant, due to the small number of populations analyzed, some spatial trends were evident (Table 7). In the first analysis, the MDH-B and MDH-C alleles gave significant individual autocorrelation coefficients, with the first DC positive and the second one highly

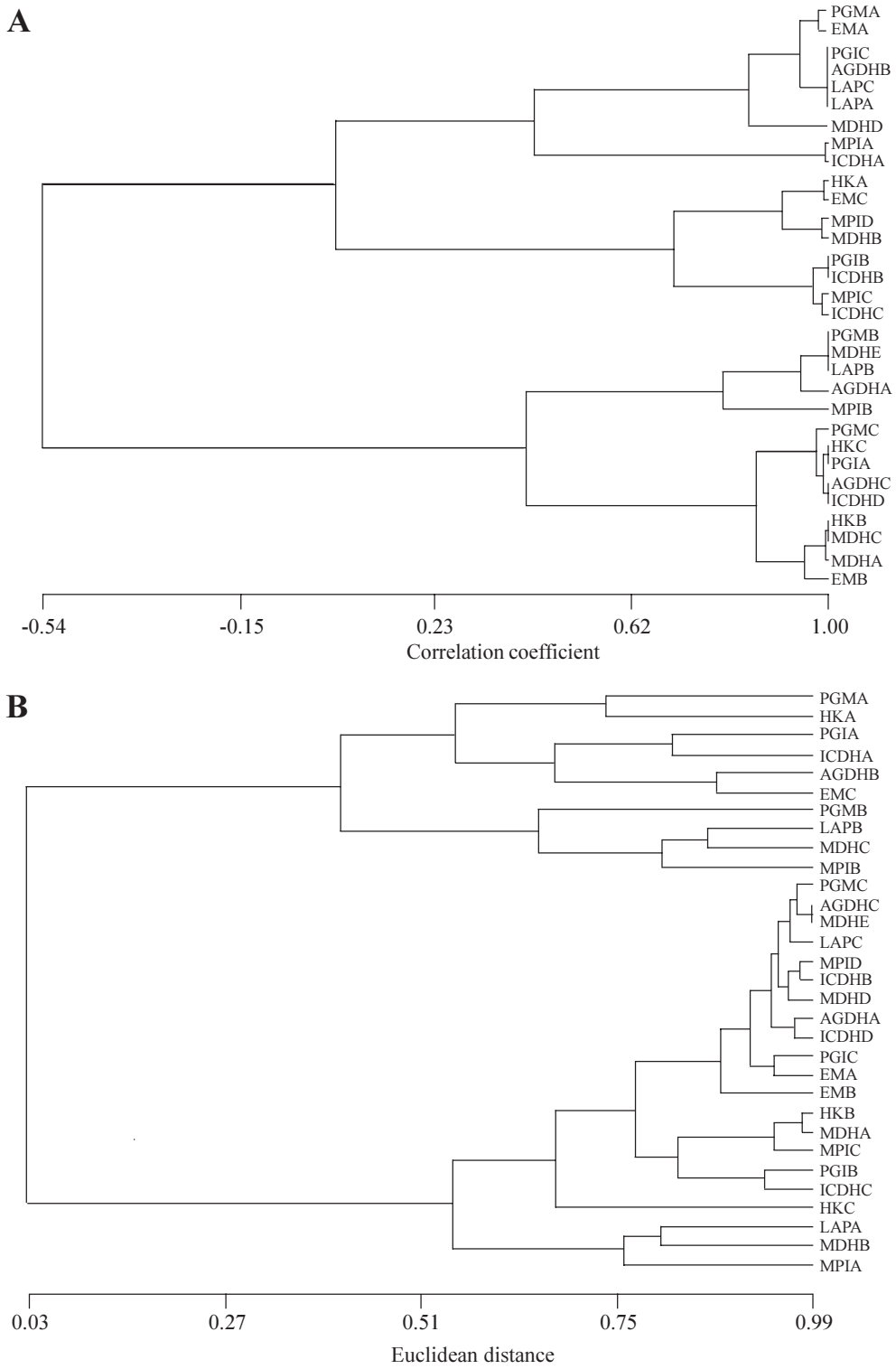

Figure 2. UPGMA trees among the 31 alleles detected in the three *Psorophora* populations studied in Colombia. A, Correlation coefficient; B, Euclidean distance.

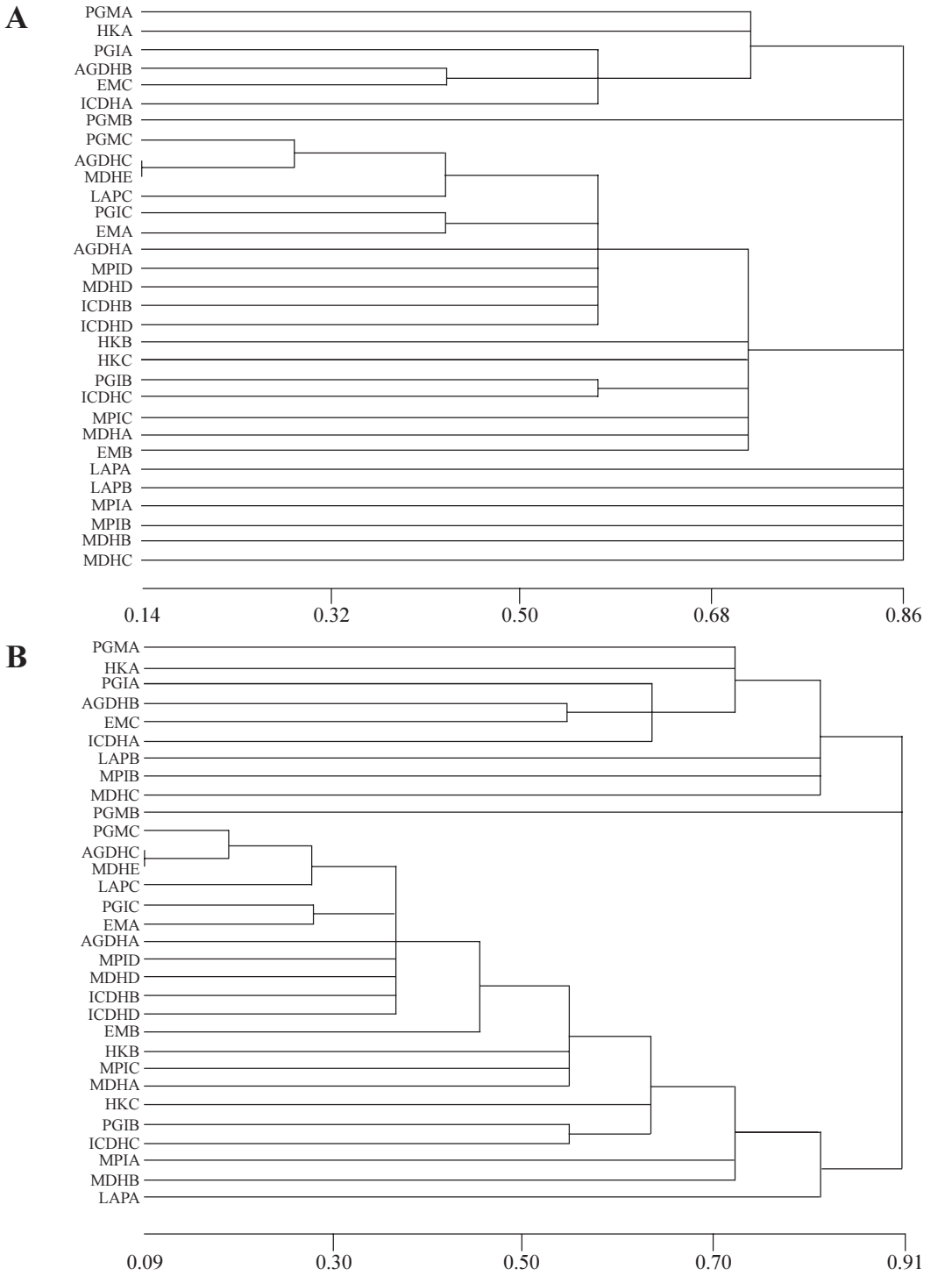

Figure 3. Consensus trees of the relationships among the 31 alleles found in nine polymorphic isoenzyme loci. A, Strict consensus between the UPGMA tree with the Euclidean distance and the neighbor-joining tree with the Euclidean distance. Colles's index = 0.34483; Mickevich's index = 0.17778; Schuh and Farris´s index = 242; B, Stinebrickner consensus (s = 0.8) between the UPGMA tree with the Euclidean distance and the neighbor-joining tree with the Euclidean distance. Colles's index = 0.51724; Mickevich's index = 0.41778; Schuh and Farris's index = 849.

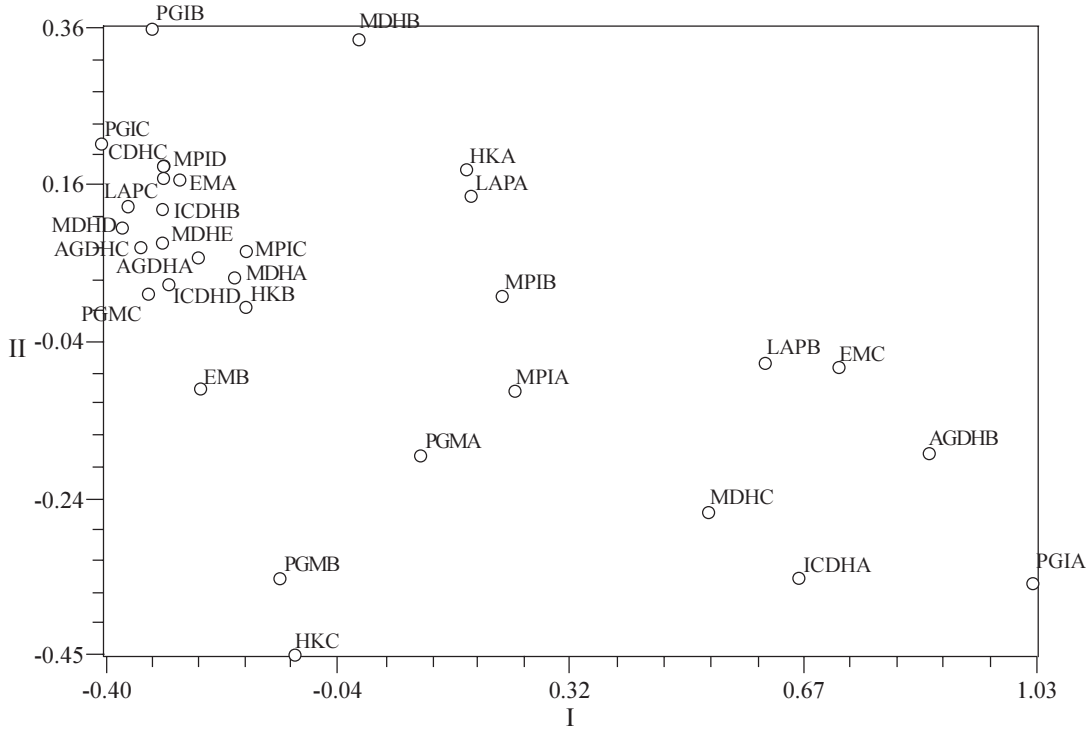

Figure 4. Nonmetric multidimensional scaling analysis with the Euclidean distance among the 31 alleles of the nine polymorphic loci analyzed for three *Psorophora* populations in Colombia. Final stress statistic = 0.03719.

Table 6. F Fisher-Snedecor pairwise tests among all variables for nine polymorphic loci of three *Psorophora* populations in Colombia.

Enzymes	χ^2	d.f.	MDH 43.512	PGM 51.072	ICDH 27.606	α-GDH 0	ME 9.612	HK 49.248
MDH	43.512	8	–					
PGM	51.072	4	1.173745 (2, 6) NS	–				
ICDH	27.606	6	1.576179 (6, 4) NS	1.850032 (2, 4) NS	–			
α-GDH	0	4	Inf (6, 2) Yes	Inf (2, 2) Yes	Inf (4, 2) Yes	–		
ME	9.612	4	4.52684 (6, 2) NS	5.313358 (2, 2) NS	2.87203 (4, 2) NS	Inf (2, 2) Yes	–	
HK	49.248	4	1.131825703 (2, 6) NS	1.037037 (2, 2) NS	1.78396 (2, 4) NS	Inf (2, 2) Yes	5.12359 (2, 2) NS	–
LAP	8.904	4	4.88679 (6, 2) NS	5.73618 (2, 2) NS	3.1004 (4, 2) NS	inf (2, 2) Yes	1.0795148 (2, 2) NS	5.530997 (2, 2) NS
PGI	39.168	4	1.110906 (6, 2) NS	1.303921 (2, 2) NS	1.4188219 (4, 2) NS	Inf (2, 2) Yes	4.074906 (2, 2) NS	1.257352 (2, 2) NS
MPI	16.848	6	2.58262 (6, 4) NS	3.031339 (2, 4) NS	1.63853 (4, 4) NS	Inf (2, 2) Yes	1.7528 (2, 4) NS	2.923076 (2, 4) NS

d.f. = degrees of freedom of the respective χ^2 test. Numbers within parentheses are the degrees of freedom for the F Fisher-Snedecor test. NS = nonsignificant difference; Yes = significant heterogeneity among loci pairs; Inf = infinite.

Table 7. Spatial autocorrelation analysis using the Moran's I index with distance classes defined with equal number of point pairs.

Alleles	Distance class 0-505 km	Distance class 677 km	Overall probability
MDH-A	0.10	-0.77*	0.365
MDH-B	0.26	-0.93*	0.100
MDH-C	0.28	-0.95*	0.102
MDH-D	0.23	-0.89*	0.272
PGM-A	0.38	-1.05*	0.181
PGM-B	0.33	-1.00*	0.220
ICDH-A	-0.61	-0.06	0.228
ICDH-C	-0.69*	0.02	0.173
ICDH-D	-0.08	-0.59	0.255
LAP-A	0.38	-1.05*	0.185
LAP-B	0.37	-1.04*	0.189
α-GDH-A	0.16	-0.082	0.327
α-GDH-B	0.34	-1.01	0.217
ME-B	0.27	-0.94*	0.259
ME-C	0.30	-0.96*	0.245
HK-A	-0.17	-0.50	0.470
HK-B	-0.42	-0.25	0.841
PGI-A	-0.25	-0.41	0.734
PGI-B	-0.69*	0.03	0.281
MPI-A	-0.56	-0.11	0.317
MPI-B	-0.15	-0.52	0.609
MPI-C	-0.77*	0.10	0.178

For abbreviations, see legend to Table 1. * Significant autocorrelation coefficient ($P < 0.05$).

negative. The MDH-A, PGM-A, PGM-B, LAP-A, LAP-B, α-GDH-A, α-GDH-B, ME-B and ME-C alleles also produced a positive first distance class (a strong relationship between neighboring populations) and a highly negative second distance class (strong differences between distant populations), although the overall correlogram was not significant. Therefore, these alleles could be adequately explained by an isolation by distance model. Nevertheless, another group of alleles yielded a negative value for the 1 DC (strong differences among the neighboring populations) and values near 0 or even slightly positive (absence of relationships) for the second DC. These alleles were ICDH-A, ICDH-C, ICDH-D, HK-A, HK-B, PGI-A, PGI-B, MPI-A, MPI-B and MPI-C. In the second spatial autocorrelation analysis, the trend was less marked than in the first analysis. Most of the alleles studied showed a positive first DC and the second DC was highly negative. The more conspicuous exceptions were ICDH-C, PGI-B and MPI-C, which gave a slightly positive first DC, whereas the HK-B allele yielded a highly negative first DC and thus behaved totally opposite to the remaining genetic markers. These alleles indicated nonexistence of overall isolation by distance among *Ps. columbiae* and *Ps. toltecum*. Despite these trends, the percentage of significant autocorrelation coefficients was not significantly different than the 5% error I margin. The Manhattan distance analysis used to detect possibly identical evolutionary spatial events affecting the isoenzymes is presented in Figure 5. The UPGMA procedure with the correlograms coming from the first spatial autocorrelation analysis showed five different spatial isoenzyme arrays: 1) MDH-B, ME-B, MDH-C,

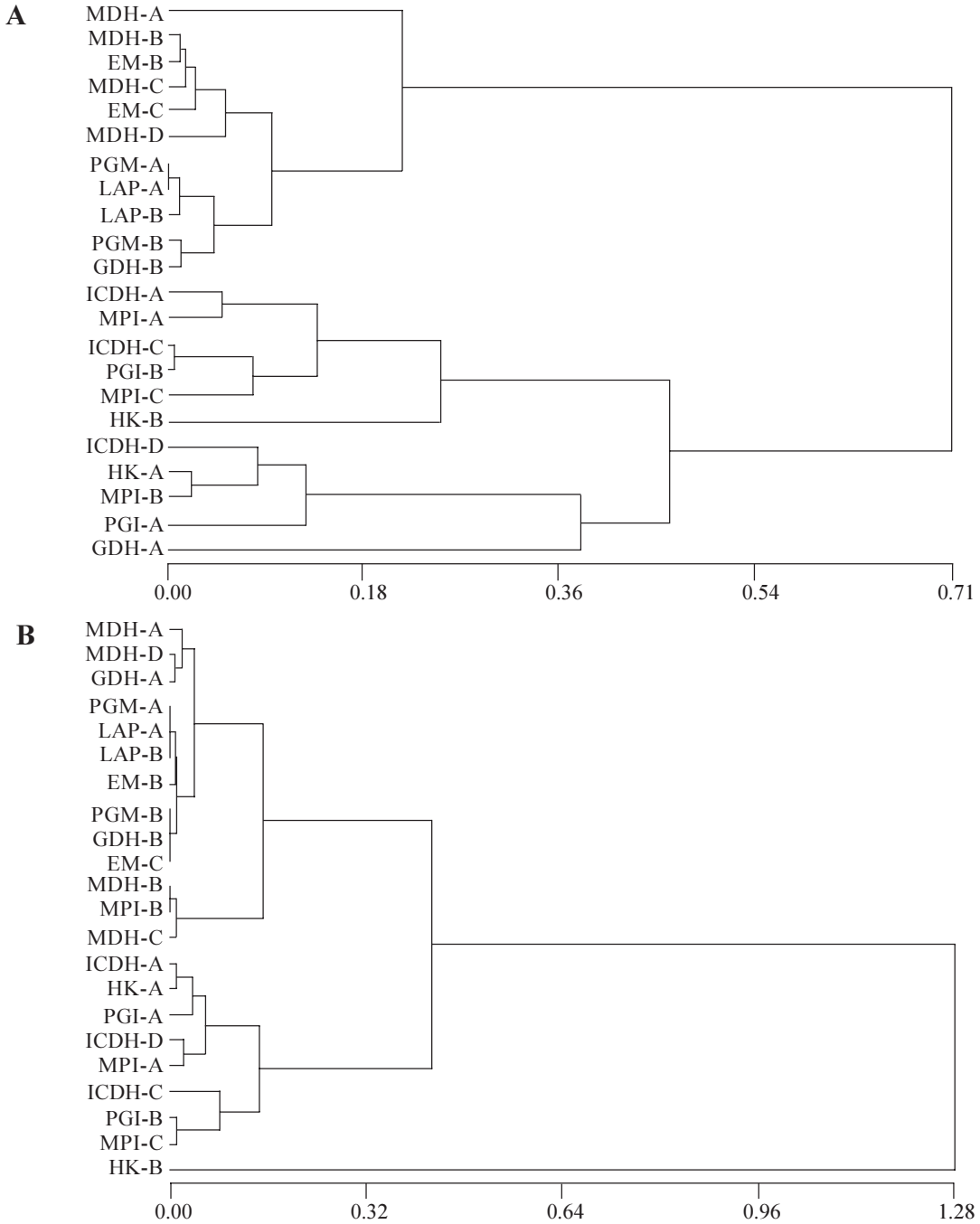

Figure 5. Trees with the Manhattan distance among correlogram pairs obtained through the Moran's I index. A, UPGMA tree having defined two distance classes (0-505 km, 505-677 km), with an equal number of relation pairs in each distance class; B, UPGMA tree having defined two distance classes (0-338 km, 338-667 km) with the same geographical size, independent of the number of relation pairs in each distance class; C, Strict consensus tree, having defined two distance classes (0-505 km, 505-677 km) with an equal number of relation pairs in each distance class. Colles's index = 0.4000; Mickevich's index = 0.33636; Schuh and Farris's index = 142; D, Strict consensus tree, having defined two distance classes (0-338 km, 338-667 km) with constant geographical sizes independent of the number of relation pairs in each distance class. Colles's index = 0.25; Mickevich's index = 0.24545; Schuh and Farris's index = 357.

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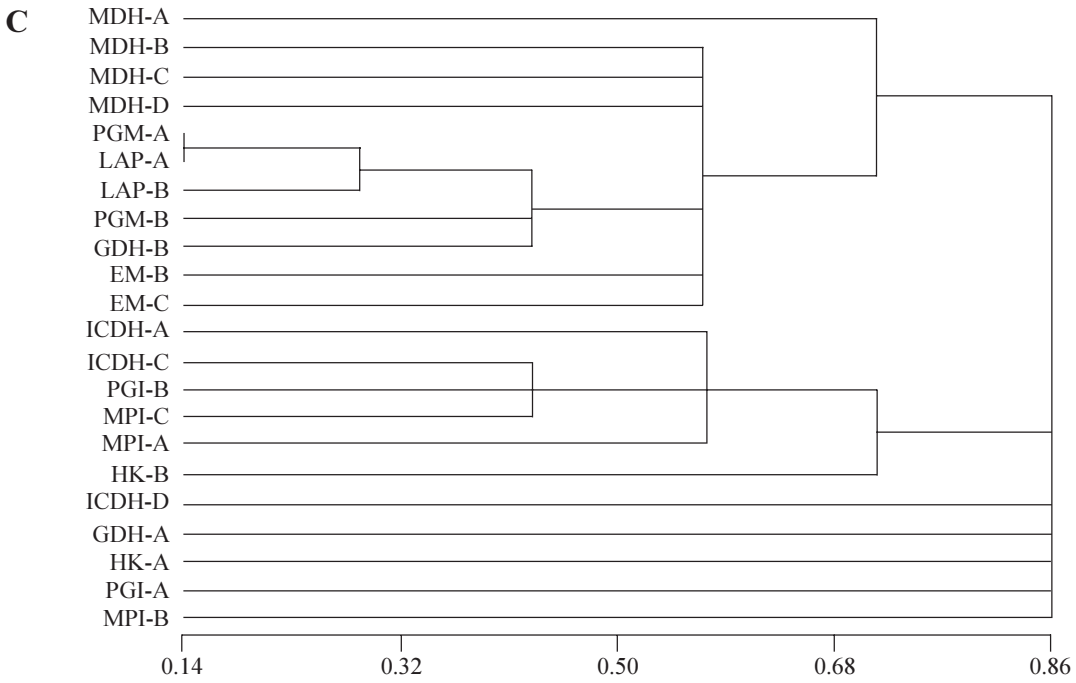
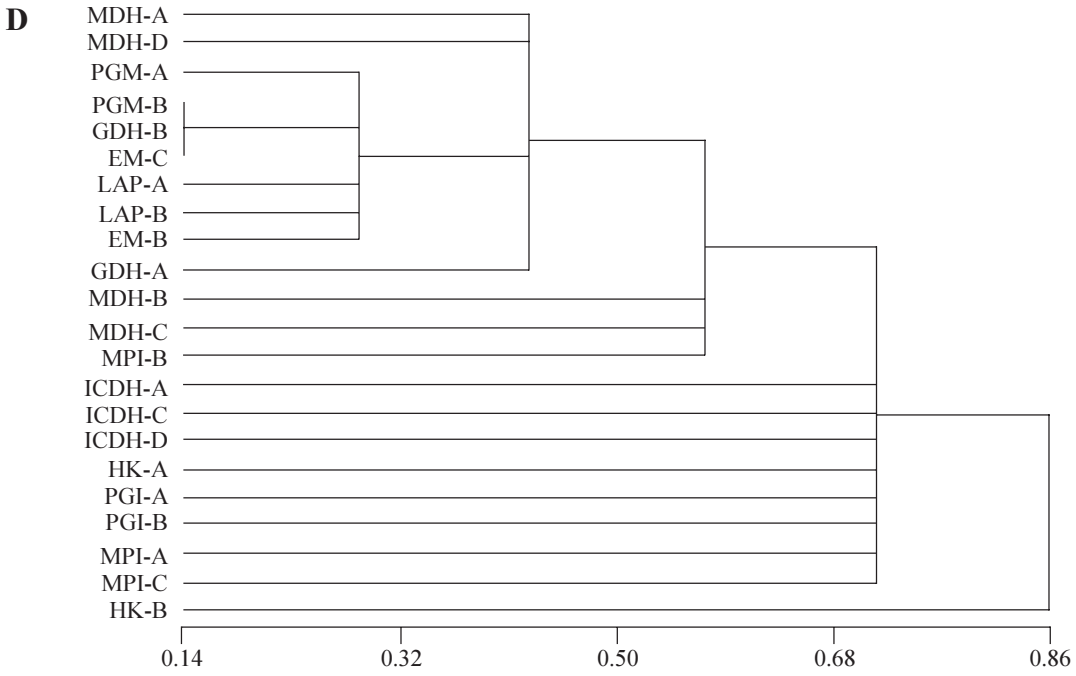
Figure 5. Continued.





ME-C and MDH-D, 2) PGM-A, LAP-A, LAP-B, PGM-D and GDH-B, 3) ICDH-A and MPI-A, 4) ICDH-C and PGI-B, and 5) ICDH-D, HK-A and MPI-B. With the second spatial autocorrelation analysis, however, only four spatial isoenzyme arrays were detected: 1) MDH-A, MDH-B, GDH-A, PGM-A, LAP-A, LAP-B, ME-B, PGM-B, GDH-B and ME-C, 2) MDH-B, MPI-B and MDH-C, 3) ICDH-A, HK-A, PGI-A, ICDH-D, MPI-A, and 4) ICDH-C, PGI-B, and MPI-C. The strict consensus tree obtained from the UPGMA and the neighbor-joining dendrograms showed several alleles in discordance depending on the tree procedure employed. These alleles were ICDH-D, GDH-A, HK-A, PGI-A and MPI-B. This means that these alleles do not have strong spatial relationships with the other alleles. The strict consensus trees obtained with the second spatial autocorrelation analysis showed that the spatial relationship among the alleles PGM-B, GDH-B and ME-C was the strongest. In the first autocorrelation spatial analysis, the percentage of correlogram variable pairs lower than 0.1 was 19.48% (45/231), which was significantly higher than the 5% error margin (χ^2 = 22.55, 1 d.f., P < 0.001). Likely, this percentage was also significant for the second spatial autocorrelation analysis, 27.71% (64/231) (χ^2 = 43.53, 1 d.f., P < 0.001). Both results indicate that a limited number of evolutionary spatial events are affecting the 31 alleles, producing four or five arrays, depending on the method employed.

Regression analysis of the genetic variability of the *Psorophora* populations and several ecological variables

Multiple regression equations among the expected heterozygosity (EH) and the average number of alleles per locus (ANAPL), and three ecological variables, including altitude (ALT), temperature (TEMP) and human population numbers (HPS) of the localities investigated were as follows: $EH = 0.7265 \times 10^{-5}$ (ALT) – 0.45×10^{-2} (TEMP) + 0.4673×10^{-6} (HPS) – 0.3054, with multiple correlation coefficient, $P^2 = 1$, and $ANAPL = 0.2782 \times 10^{-2}$ (ALT) – 0.07875 (TEMP) – 0.8455×10^{-6} (HPS) + 19.44, with multiple correlation coefficient, $P^2 = 1$.

Therefore, the P^2 coefficient is equal to 1 for both genetic diversity measures. Thus, the genetic variability is totally determined for these three ecological variables. The linear, logarithmic, exponential and power equations among average expected heterozygosity and the average number of alleles per locus correlated with altitude, temperature and human population numbers are given separately (Table 8). Heterozygosity was strongly related to altitude. Altitude determined from 47.41% (linear model) to 69.27% (power model) of the expected heterozygosity. The relationship among temperature and heterozygosity was even stronger, although inversely. Temperature determined from 75.51% (linear and exponential models) to 76.39% (logarithmic and power models) of the expected heterozygosity. However, the strongest relationship found was detected between human population size and expected heterozygosity. This variable inversely determined from 95.96% (power model) to 98.70% (logarithmic model) of the expected heterozygosity. However, the average number of alleles per locus was less highly related to these three variables, independently. The strongest relationship was with human population size (ranged from 30.38% (linear model) to 62.63% (power model)), whereas the weakest relationship was with altitude (ranged from 0.8%, logarithmic model, to 1.52%, power model).

Cell line

The *Ps. columbiae* cell line, which originated from a Granada colony established two

Table 8. Simple regression analysis for total heterozygosity and average number of alleles per locus for altitude, temperature and human population size where mosquitoes were sampled.

	Linear $y = m(x) + b$	Exponential $y = m(e^{(bx)})$	Logarithmic $y = b + m\ln(x)$	Power $y = m(x^b)$
Total heterozygosity *vs* altitude				
r	0.6885	0.8266*	0.6959	0.8323*
m	8148.3207	122.9545	3352.2259	3856×10^{10}
b	-3122.8111	0.0001	3208.3946	50.3928
Total heterozygosity *vs* temperature				
r	-0.8690*	-0.8690*	-0.8740*	-0.8740*
m	-34.7205	-1.2627	-14.2140	17.1745
b	41.4750	45.7073	14.5602	-0.5169
Total heterozygosity *vs* human population size				
r	-0.9923*	-0.9816*	-0.9935*	-0.9796*
m	-260121×10^{10}	-83.8840	-10.6007	0.0000
b	1101477.6280	1923663×10^{10}	-910605.6882	-34.0738
Average number of alleles per locus *vs* altitude				
r	0.1033	-0.1097	0.0896	-0.1234
m	115.7139	-1.5444	264.8745	7430.5627
b	-112.5705	5107.9180	-63.5031	-4.5864
Average number of alleles per locus *vs* temperature				
r	0.1889	0.1889	0.2025	0.2024
m	0.7142	0.0259	2.0206	25.4308
b	25.4285	25.5002	25.3536	0.0734
Average number of alleles per locus *vs* human population size				
r	0.5512	0.7829	0.5627	0.7914
m	136699.3607	6.3292	368373.9163	0.0018
b	-322528.6285	0.0013	-318900.5294	16.8901

r = correlation coefficient, m = slope, b = coefficient. * Significant correlation coefficient ($P < 0.05$).

years previously in the lab, was compared with isoenzymatic data from the mosquito sampled from the same wild population directly (Table 9). Electrophoretic analyses showed that wild-caught individuals had a higher number of genotypes than those from the cell line.

DISCUSSION

Polymorphisms, number of alleles per locus and genetic variability

Isoenzyme polymorphism in the three populations of *Psorophora* was high. Nine of the 11 markers were polymorphic (82%). No previous data exist for *Psorophora* in South America. In other studies, similar, even slightly lower, percentages have been found, such as in Dujardin et al. (1996) with *Lutzomyia*, who found that five of seven markers were polymorphic (71%), Byrne and Nichols (1999) with *Culex pipiens* (90%), Le Pont et al. (1985) with *P. carrerai*, Caillard et al. (1986) with *P. yucumensis*, and the study of *Phlebotomus papatasi* carried out by Kassem et al. (1990). Nevertheless, the number of alleles per locus found in our study (2.5-2.8) was higher than that reported for other species, such as *Lutzomyia trapidoi*

Table 9. Genotypes of wild-caught individuals and genotypes of a cell line cultivated from the Granada population of *Psorophora columbiae* in Colombia.

Enzyme	Wild caught	Cell line
MDH	ac, bb, cc, aa, ee, ad, bc	ad
PGM	ab, aa, cc, bc	aa
ICDH	aa, ad, ac, bd	aa
6-PGDH	aa	aa
α-GDH	ab, bc, bb	ab
ME	cc, ac, bc	cc
HK	aa, bb, bc, ac	aa
LAP	aa, ab, bb	ab
PGI	aa, ab	aa
MPI	aa, bb, bc, bd, ac	ad
ASAT	aa	aa

ASAT = aspartate transaminase ; 6PGDH = 6-phosphogluconate dehydrogenase. For other abbreviations, see legend to Table 1.

(1.57-2.29) (Dujardin et al., 1996), but similar to that determined by Kassem et al. (1993) for *Phlebotomus papatasi*. In other insects, the percentage of polymorphism was much lower. Several examples include: *Lutzomyia* sp. in Colombia (19%) and *Lutzomyia andina* (47.4%) (Kreutzer et al., 1990, who studied seven species of the verrucarum group), or *Neodiprion* (8.8-34.8%; Woods and Guttman, 1987). This could be motivated by the selection of the markers in this study as there was a skew in favor of markers that had indicated polymorphism in another organisms. The level of expected heterozygosis for each of the populations was high (0.39-0.42) for this kind of marker, although slightly lower than that found with other more recently employed molecular markers. Several examples are the case of *An. gambiae* in Kenyan populations using microsatellite markers (H = 0.59-0.66) (Lehmann et al., 1998), as well as the intra-population haplotype mtDNA diversity in populations of *An. rangeli* and *An. trinkae* in Bolivia (H = 0.717-1.000; H = 0.822-0.867), and in Ecuador (H = 0.667-1.000; H = 0.707), respectively (Conn et al., 1997). Nonetheless, with the same mtDNA markers, other mosquito species have shown haplotype diversities lower than the nuclear gene diversity indicated here. This was the case for *An. aquasalis* (H = 0.219; Conn et al., 1993) and *A. quadrimaculatus* species A (H = 0.370; Perera et al., 1995). As there was a strong degree of nuclear gene diversity in the *Ps. columbiae* and *Ps. toltecum* populations and their respective distribution ranges are large, it is certainly probable that the mtDNA diversity in these mosquito species would be high as well. This aspect remains to be studied. In contrast, other studies with other diptera have found heterozygosity values slightly, or much, lower that those we found with isoenzyme markers. Kreutzer et al. (1990) found a range of H = 0.082-0.171 for several species of *Lutzomyia*, whereas H was 0.12-0.145 for *L. carrerai* and *L. yucumensis* (Caillard et al., 1986), 0.036 for *L. longipalpis* (Bonnefoy et al., 1986) and 0.009-0.028 for *E. solidaginis* (Waring et al., 1990). The fact that the two *Psorophora* populations had very similar levels of (high) genetic variability could be attributed to the antiquity of these species in these localities or elevated gene flow among these two *a priori* species. It is recognized that newly colonized areas normally have less genetic diversity compared with the original or ancestral populations (Avise et al., 1987).

Hardy-Weinberg equilibrium and genetic distances

By using the results obtained with the f of Robertson and Hill (1984), strong deviations from HWE were detected for homozygous excess as well as for heterozygous excess, at least for one population, although the homozygous excess predominated. These deviations were present in all three populations, with the Ambalema population having the largest number of genetic markers in HW disequilibrium, simultaneously showing homo- and heterozygous excess. Overall, the deviations mainly affected three loci: PGI, HK and MPI. Relative similar results have been found for other insects, such as *Ps. papatasi* (Kassen et al., 1993). The inexistence of panmixia and higher amounts of gametic disequilibrium, as well as the presence of a diagnostic locus (GPD), were valuable insights, which led Dujardin et al. (1996) to determine the existence of at least two strongly related species of *Lutzomyia* in Paraiso Escondido and La Tablada (Ecuador). In fact, in this last case, none of the markers were in HWE, with the exception of GPI. Likely, several markers, such as 6-PGDH, FUM, PFK and PGM would be useful to determine and discriminate new *Lutzomyia* species in Colombia, such as *L. townsendi* and *L. longiflocosa* (Kreutzer et al., 1990).

Several authors, such as Ahmad et al. (1980) and Ruiz-Garcia (1994a), showed that strongly related populations, from a population genetics point of view, gave a Nei's genetic distance (Dn) < 0.02, while less strongly related populations gave values of 0.08 > Dn > 0.02, and the hardly divergent populations yielded Dn values > 0.1. We found that the Nei's genetic distances among the populations of Lorica (Colombian Atlantic coast, apparently *Ps. toltecum*) and the Andean region at the center of the country (Meta and Tolima, apparently *Ps. columbiae*) were relatively small, especially for the pair Ambalema-Lorica (D = 0.0438), and slightly higher for Granada-Lorica (D = 0.0814). These distances were smaller than those found by Ayala (1975) for subspecies of *Drosophila* (D = 0.15-0.18) and than that found in incipient species (D = 0.19-0.21). Indeed, the Nei's distance among cryptic species of *L. trapidoi* was 0.211, higher than that found among the two *Psorophora* taxa that we studied. In fact, genetic differences among different Venezuelan and Trinidadian populations of *An. aquasalis* and among the four sibling species of *An. quadrimaculatus* (Mitchell et al., 1992) were higher than that detected in our study. However, other morphologically similar species yielded much higher Nei's genetic distance than those indicated here. This is the case for *Ae. dorsalis* and *Ae. melanimon* (D = 0.825) and *Ae. campestris* versus *Ae. canadensis* (D = 0.90) (Gimnig and Eldridge, 1999).

Lanzaro (1997) analyzed several USA (Lousiana, Mississipi, Alabama, Texas, Florida, Maryland, Georgia, Arkansas), Mexican (Corral Nuevo, Veracruz) and three Colombian *Ps. columbiae* populations. The North American populations were highly similar to the Colombian populations (average value of D = 0.014; oscillation range D = 0.001-0.052). On the other hand, in this same analysis, several USA (Texas, Arizona and California) and Mexican (Tehuantepec) *Ps. toltecum* populations showed a Nei's genetic distance compared to *Ps. columbiae* of around 0.208-0.360 with a mean of 0.277, which is substantially higher than what we found in this study. Among the four *Ps. toltecum* populations studied by Lanzaro (1997), the Nei's genetic distance ranged from 0.01 to 0.071 with a mean of 0.039. That is, the Eastern USA populations (*Ps. columbiae*) and the Western USA populations (*Ps. toltecum*) had greater genetic differences than that found among the Central Colombian Andean *Ps. columbiae* populations and the Atlantic Colombian Coast population of *Ps. toltecum*. This could mean that only one species was actually analyzed in Colombia (*Ps. columbiae*) and the presumed *Ps. toltecum*

from the Atlantic Colombian coast is not a different species from that of the populations sampled in the Colombian Andean area. Nevertheless, the genetic distance values obtained were greater than those found by Lanzaro (1997) among strictly Colombian and USA *Ps. columbiae* populations, which also raises the possibility that actually two species were sampled in Colombia but with a relatively strong gene flow between them. The genetic distance between these species and the true *Ps. confinnis* from Argentina ranges from 0.251 to 0.289 (mean, 0.263) with *Ps. columbiae* and from 0.293 to 0.385 (mean, 0.333) with *Ps. toltecum*. Therefore, our results indicate that these populations are not closely related to *Ps. confinnis*.

It is clear that multi-locus enzyme profiles are a useful tool for the detection of cryptic species, although in our study there were relatively few differences. Using this procedure, *Ae. tahoensis* was differentiated from *Ae. communis* (Brust and Munstermann, 1992), *Ae. increpitus* was described as a species complex with three cryptic species (Lanzaro and Eldridge, 1992), and *Ae. dorsalis*, previously considered as a subspecies of *Ae. caspius*, was determined to be a reproductively isolated taxa in sympatry with the latter species (Lambert et al., 1990). Nevertheless in our study, the isoenzyme profiles did not have enough discriminating power to differentiate between the *Psorophora* populations.

Although the genetic distances and the F_{ST} statistics did not reveal striking differences between the two supposed *Psorophora* species, several exclusive alleles were found in the two taxa.

We found cases of heterozygous excess for certain markers, as well as heterozygous excess for other loci. These results are not easy to explain. In other species, such as *P. mixtum*, homozygous (MDH-2 and LDH) and heterozygous excess (ICDH and PGI) have also been found simultaneously. Endogamy should act in an identical way on the complete genome and the homozygous excess should therefore be general for all the markers. This occurred in Lorica and in Granada, but not in Ambalema, nor was it true for all populations considered together. However, several markers, such as MDH, ME or LAP, were generally in HWE, whereas others yielded a clear homozygous excess, which is not compatible with generalized endogamy. A second possibility is the existence of differential selective patterns affecting several markers associated with different habitats. If differential selective forces were acting upon only some of the markers, Wahlund effect would be present, which could explain the excess homozygotes in loci such as α-GDH, HK and MPI. Additionally, there could be heterotic selection (selection in favor of heterozygotes) affecting the PGM locus in all the populations. While certain diptera species have a reduced dispersion capacity and long generation times (Morrison et al., 1993), which could exclude the existence of a Wahlund effect, in other diptera species, such as *Psorophora*, dispersion throughout diverse neighboring populations is elevated, which could provoke a Wahlund effect at the time of sampling. An alternative scenario could be as follows: The greatest genetic distances were found among Granada and Lorica (supposedly *Ps. columbiae* versus *Ps. toltecum*) and among Ambalema and Granada (two supposed populations of *Ps. coumbiae* separated by the Eastern Andean mountains), whereas the smallest distance was detected between a central Andean population, Ambalema and the Atlantic coast *Ps. toltecum* population. In the Lorica and in the Granada population, the HW deviations were low and always had homozygous excess. This could be caused by the Wahlund effect in those loci exposed to diversifying natural selection in these areas, or simply by stochastic causes. In contrast, the Ambalema population showed high levels of HW deviations, with simultaneous homo- and heterozygous excess. This could mean that Ambalema was influenced by gene flow and

migration from Lorica or nearby populations. Only a fraction of these mosquitoes crossed with the original Ambalema population and generated excess heterozygotes at several loci (those in which the two species had different allele frequencies), whereas another portion never really crossed with the Ambalema population and caused a positive Wahlund effect. This would have occurred more frequently in Ambalema than in the other populations. Lanzaro (1997) detected important hybrid areas in USA and Mexico between these two *Psorophora* species. Another possibility, undoubtedly less parsimonious, would be assortative mating for several biochemical markers. However, it is not likely that all the loci would yield excess homozygotes independently, linked to different morphological traits, or that they were all linked.

The potential influence of the population structure on speciation could be very important (Templeton, 1980). If speciation occurred allopatrically, following a vicariant event, the genetic structure of the original population would not be very important. Nevertheless, if the speciation phenomenon occurred in sympatry, the original genetic structure would be very important. If the original taxa had a wide geographic distribution and their effective numbers were elevated, the species, or taxa, could have provided most of the genetic variability of the ancestral taxa. Lorica population (*Ps. toltecum*), which differed only slightly from the other two populations (*Ps. columbiae*), could have originated in this way, or vice versa.

Evolutionary relationships among the alleles

It is interesting to note that the allele relationships were different, depending on whether correlation coefficients or the Euclidean distance was employed. This means that the mathematical properties of each statistic have a substantial influence on the results. On the other hand, it could mean that the differences between the alleles of different loci were small.

One could expect *a priori* that the allele frequencies among alleles at the same locus would be more related than the allele frequencies among different loci. However, this is not true. Only the alleles LAP-C and LAP-A, ICDH-B and ICDH-C, and MDH-A and MDH-C, belonging to the same locus, were highly related. In contrast, it was more frequently found that diverse alleles at a given locus were in different allele clusters. The PGM-A allele was found in subcluster 1 of the first main UPGMA cluster, while the PGM-B and the PGM-C alleles were in subclusters 1 and 2 of the second main cluster, respectively. The same was observed for the PGI alleles. The PGI-C allele was in subcluster 1 of the first cluster, whereas PGI-B and PGI-A alleles were in subcluster 2 of the first cluster and in subcluster 2 of the second main cluster, respectively.

Genetic heterogeneity, spatial structure and gene flow

The genetic structure of the three populations from the two supposed taxa was significant but relatively small ($F_{ST} = 0.067$). It was found that there were markers that discriminated somewhat at an intrapopulation level (PGM, α-GDH and MPI), and others that strongly discriminated at an interpopulation level (HK, PGI and PGM). The markers which best discriminated at both levels were α-GDH, HK, PGM and MPI.

Other insect populations have been found with similar genetic differentiation levels. Several cases are as follows: *An. gambiae* showed, in two Kenyan populations (Jego and

Asembo), a value of F_{ST} = 0.072-0.10 (Lehmann et al., 1998), although the genetic differentiation was conspicuously lower in other African areas separated by 6,000 km (F_{ST} = 0.016; Lehmann et al., 1996). Other species, such as *Prosimulium mixtum*, yielded a value of F_{ST} = 0.096, even though *P. fuscum* had much lower genetic heterogeneity (F_{ST} = 0.003).

The three loci, which yielded the highest F_{ST} values were PGM, HK and PGI (0.133, 0.171 and 0.136, respectively). It is possible that diversifying natural selection was acting upon these loci more intensively than upon the remaining loci, whose dynamics were more neutral. The existence of differentiated habitats was the explanation invoked by Lehmann et al. (1998) to understand the striking genetic differences among Kenyan populations separated by 700 km, with arid valleys, mountains and dry savanna regions, which were effective barriers to gene flow. Contrary to these ecological and climatic explanations, the genetic evidence we found seems to indicate inexistence of restrictions to gene flow among the *Psorophora* populations from the Colombian Atlantic coast to the central Andean area of Colombia. The inexistence of isolation by distance (Slatkin, 1993) and the diverse opposite trends for the different isoenzyme loci analyzed by spatial autocorrelation agree quite well with an absence of restriction to gene flow among the Atlantic and central Andean populations and/or that this divergence is very recent. Nevertheless, whereas some generalist Diptera species, such as *Zaprionus indianus* in India (Parkash and Yadav, 1993) and *Drosophila subobscura* in Europe (González-Duarte et al., 1993), showed a latitudinal clinal divergence, in our case the geographical differences found, more than the clinal patterns, could be a consequence of relatively strong gene flow that did not follow determined routes.

If these two *Psorophora* taxa are indeed species differentiated a long time ago, we expect that the historical estimate of Nm would be around or below one, which is considered the minimal value for compensating the action of gene drift and gene flow. Obviously, it is assumed that $\underline{m}$ is high enough above the mutation rate per generation (μ). Nevertheless, the gene flow estimates among all the populations analyzed were relatively elevated (Nm = 3.48 for the infinite island model and Nm = 2.00-2.17 for the private allele model), similar to those reported among Kenyan *Anopheles gambiae* populations (Nm = 3.2-3.3; Lehmann et al., 1998). The unique genetic differences found among Lorica and the other two populations were characteristic alleles at the α-GDH, MDH, ME, PGI and LAP loci, at relatively low frequencies. A similar pattern was detected for *An. rangeli*, whose populations in four South American countries displayed unique haplotypes probably due to isolation by distance (Conn et al., 1997). In our case, the gene flow among the populations is apparently relatively extensive, being impossible to distinguish if the detected gene flow occurs currently or if the genetic similarity is a product of a relatively short time of divergence between the two taxa. For instance, in several mosquito species living in sympatry, including *A. rangeli* and *A. trinkae*, Conn et al. (1997) did not detect any evidence of hybridization in the form of shared mtDNA haplotypes. Gene flow, therefore, seems to be important for two supposedly different species. That means that gene flow is relatively high for an island model, independent of the population genetics procedure employed. It would be an indication of a single *Psorophora* species in the Colombian area we investigated. When the isolation by distance test created by Slatkin (1993) was applied to our data, no evidence was detected, which led us to put in doubt again the possibility that the central Colombian *Psorophora* was really a different species from the Atlantic Colombian population.

Gene flow data can be employed to help determine the timing and breadth of insecticide

treatment for vector control (Tabachnick and Black, 1995). The apparently unrestricted gene flow between *Ps. columbiae* and *Ps. toltecum* could mean that insecticide treatment control should not be independent and local. In addition, both species have relatively high genetic diversities and an inverse relationship between genetic diversity and the success of vector control strategies in African vector mosquitoes has been demonstrated. Therefore, general and intensive insecticide treatment controls must be taken into account to diminish the probability of expansion of VEE.

We found extremely low (0.804 and 0.647), intermediate (3.094, 6.364, 8.324, 9.258), and extremely high (84.687 and infinite) gene values among the same population pair, depending on the isoenzymes employed. This could be evidence that the evolutionary trends of each of the isoenzymatic markers are differentially affected by different forces.

Sokal et al. (1986, 1989) showed by means of simulations that correlogram pairs generated by the same evolutionary spatial processes have Manhattan distances smaller than 0.1 for the Moran's I index. If the percentage of Manhattan distances smaller than 0.1 is significantly superior to the 5% error margin, then we can claim the existence of one, or a limited number of spatial evolutionary events affecting the isoenzyme variables studied. Effectively, four or five spatial allele clusters were detected, depending on the procedures employed. This means that in studies of this nature it is important to analyze a considerable set of biochemical markers that could be affected differentially by several spatial evolutionary trends.

Genetic variability and relationships with ecological parameters

It clearly seems that overall heterozygosity is more determined by temperature, altitude and human population size than the average number of alleles per locus did.

We noted striking relationships, especially among the degree of heterozygosity and these three variables, which could be an indication that natural selection acted upon the isoenzyme markers in an overall way. It would be interesting to analyze the *Psorophora* genetic characteristics in other American countries to determine if there is strong geographic partitioning by country, such as has been found for *An. rangeli* in Bolivia, Ecuador and Venezuela, or if the same genetic variants are dispersed across all populations studied, such as the case for *An. trinkae* in Bolivia and Ecuador (Conn et al., 1997).

Cell line

Cell line genotypes of *P. columbiae* established from embryo tissues from a captive colony surveyed originally from Granada were compared with those obtained directly from nature. The results indicated cell line fixation and loss of alleles at several loci. There was a loss of genetic variability in the cell line by fixation of alleles of the PGM, ICDH, 6-PGDH, EM, HK, PGI and ASAT loci. These results can be interpreted in different ways. 1) It is possible that due to gene drift during the sampling process, a lot of different genotypes were lost; 2) the original natural population sampled in Granada during 1997 to establish the captive colony was slightly different from the population sampled in our study. For instance, this hypothesis is sustained by the presence of a genotype (ad) at the MPI locus in the cell line, which was not found in the natural population; 3) possible changes induced by endogamy and gene drift during

the process of cell line constitution in the laboratory. Morrison et al. (1993), in a comparative study of *L. longipalpis* in Melgar (Colombia), found genetic differences between laboratory cultures and individuals collected directly from nature. Mukhopadhyay et al. (1997) also compared the genetic profiles of five laboratory colonies of this species, from Brazil and Colombia, to natural populations from the same localities. There is loss of alleles and reduced heterozygosity in the lab colonies, as a consequence of the colonization process. 4) During the cell line formation process, several selective forces changed relative to those present in the wild. These selective forces could have fixed determined genotypes, decreasing the genetic variability.

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REFERENCES

Ahmad, M., Blumenberg, B. and **Chaudhary, M.F.** (1980). Mutant allele frequencies and genetic distance in cat populations of Pakistan and Asia. *J. Hered. 71*: 323-330.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. and **Saunders, N.C.** (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu. Rev. Ecol. Syst. 18*: 489-522.

Ayala, F.J. (1975). Genetic differentiation during the speciation process. *Evol. Biol. 8*: 1-78.

Barton, N.H. and **Slatkin, M.** (1986). A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity 56*: 409-416.

Bloem, S. (1990). Enzymatic variation in the Varipalpus group of *Aedes* (*Ochlerotatus*) (Diptera: Culicidae). *Ann. Entomol. Soc. Am. 84*: 217-227.

Bonnefoy, D., Tibayrenc, M., LePont, F., Dujardin, J.P., Desjeux, P. and **Ayala, F.J.** (1986). An isozymic study of *Lutzomyia longipalpis* (Diptera: Psychodidae), the vector of visceral leishmaniasis in the "Yungas" (Bolivia). *Cah. O.R.S.T.O.M. Ser. Entomol. Med. Parasitol. 24*: 213-217.

Brust, R.A. and **Munstermann, R.K.** (1992). Morphological and genetic characterization of the *Aedes* (*Ochlerotatus*) *communis* complex (Diptera: Culicidae) in North America. *Ann. Entomol. Soc. Am. 85*: 1-10.

Byrne, K. and **Nichols, R.A.** (1999). *Culex pipiens* in London underground tunnels: differentiation between surface and subterranean populations. *Heredity 82*: 7-15.

Caillard, T., Tibayrenc, M., Le Pont, F., Dujardin, J.P., Desjeux, P. and **Ayala, F.J.** (1986). Diagnosis by isozyme methods of two cryptic species, *Psychodopygus carrerai* and *P. yucumensis* (Diptera: Psychodidae). *J. Med. Entomol. 23*: 489-492.

Cavalli-Sforza, L.L. and **Edwards, A.W.F.** (1967). Phylogenetic analysis: Models and estimation procedures. *Am. J. Hum. Genet. 19*: 233-257.

Conn, J., Cockburn, A.F. and **Mitchell, S.E.** (1993). Population differentiation of the Malaria vector *Anopheles aquasalis* using mitochondrial DNA. *J. Hered. 84*: 248-253.

Conn, J.E., Mitchell, S.E. and **Cockburn, A.F.** (1997). Mitochondrial DNA variation within and between two species of Neotropical Anophelinae mosquitoes (Diptera: Culicidae). *J. Hered. 88*: 98-107.

Coosemans, M., Smits, A. and **Roelants, P.** (1998). Intraspecific isoenzyme polymorphism of *Anopheles gambiae* in relation to environment, behavior, and malaria transmission in southwestern Burkina Faso. *Am. J. Trop. Med. Hyg. 58*: 70-74.

Craig, G.B. and **Hickey, W.A.** (1965). Genetics of *Aedes aegypti.* In: *Genetics of Insect Vectors of Disease* (Wright, J.W. de and Pal, R., eds.). Elsevier, Amsterdam, The Netherlands, pp. 67-131.

Dickerman, R.W., Cupp, E.W., Groot, H., Alarcon, A.M., Cura, E., Dickerman, A.W., Ibagos, A.L., Rico-Hesse, R., Taylor, C.A. and **Weaver, S.C.** (1986). Venezuelan equine encephalitis virus activity in northern Colombia during April and May 1983. *Bull. Pan. Am. Health Organ. 20*: 276-283.

Dujardin, J.P., Le Pont, F., Cruz, M., Leon, R., Tarrieu, F., Guderian, R., Echeverria, R. and **Tibayrenc, M.** (1996). Cryptic speciation in *Lutzomyia* (*Nyssomyia*) *trapidoi* (Fairchild & Hertig) (Diptera: Psychodidae) detected by multilocus enzyme electrophoresis. *Am. J. Trop. Med. Hyg. 54*: 42-45.

Faran, M.E. (1980). Mosquito studies (Diptera: Culicidae) 34: a revision of the Albimanus section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contrib. Am. Entomol. Inst* (*Ann Arbor*) *15*: 1-215.

Gimnig, J.E. and **Eldridge, B.F.** (1999). Genetic and morphological characterization of the *Aedes* (*Ochlerotatus*) *dorsalis* (Diptera: Culicidae) group in North America. *J. Med. Entomol. 36*: 685-694.

Gimnig, J.E., Reisen, W.K., Eldridge, B., Nixon, K. and **Schutz, S.J.** (1999). Temporal and spatial genetic variation within and among populations of the mosquito *Culex tarsalis* (Diptera: Culicidae) from California. *J. Med. Entomol. 36*: 23-29.

González-Duarte, R., González Izquierdo, M.C. and **Prevosti, A.** (1993). Polymorphism for esterases and alcohol dehydrogenases in natural populations of *Drosophila subobscura*. *Atti Accad. Sci. Ist. Bologna Rc. Cl. Sci. Fis. 261*: 63-70.

Goudet, J. (1995). FSTAT, a program for IBM PC compatibles to calculate Weir and Cockerham's (1984) estimators of F-statistics (version 1.2). *J. Hered. 86*: 344-345.

Hedrick, P.W. (1985). Coat variants in cats. Gametic disequilibrium between unlike loci. *J Hered 76*: 127-131.

Hribar, L. (1995). Coastal wing spot variation within and among progeny of single female *Anopheles nuñeztovari* (Diptera: Culicidae). *Mosq. Syst. 27*: 1-15.

Kassem, H.A., Abdel Sattar, S., Shehata, M.G. and **El Sawaf, B.M.** (1990). Electrophoretic comparison of the *Leishmania* vectors *Phlebotomus papatasi* and *Phlebotomus langeroni* (Diptera: Psychodidae). *J. Med. Entomol. 27*: 592-601.

Kassem, H.A., Fryauff, D.J., Shetata, M.G. and **Sawaf, B.M.** (1993). Enzyme polymorphism and genetic variability of one colonized and several field populations of *Phlebotomus papatasi* (Diptera: Psychodidae). *J. Med. Entomol. 30*: 407-413.

Kreutzer, R.D. and **Christensen, H.A.** (1980). Characterization of *Leishmania* spp. by isozyme electrophoresis. *Am. J. Trop. Med. Hyg. 29*: 199-208.

Kreutzer, R.D., Palau, M.T., Morales, A., Ferro, C., Feliciangeli, D. and **Young, D.G.** (1990). Genetic relationships among phlebotomine sand flies (Diptera: Psychodidae) in the *verrucarum* species group. *J. Med. Entomol. 27*: 1-8.

Kruskal, J.B. (1964). Nonmetric multidimensional scaling: a numerical method. *Psykometrika 29*: 28-42.

Lambert, M., Pateur, N., Rioux, J.A., Delalbre-Belmonte, A. and **Balard, Y.** (1990). *Aedes caspius* (Pallas 1771) et *A. dorsalis* (Meigen, 1830) (Diptera: Culicidae). Analyses morphologique et genetique de deux populations sympatriques. Preuves de l'isolament reproductif. *Ann. Soc. Entomol. France 26*: 381-398.

Lanzaro, G.C. (1997). *Genetics of the Mosquito Vector of Epidemic Venezuelan Equine Encephalitis*. *Advanced Research Program*. Texas A&M University, Houston, TX, USA.

Lanzaro, G.C. and **Eldridge, B.F.** (1992). A classical and population genetic description of two new sibling species of *Aedes* (*Ochlerotatus*) *increpitus* Dyar. *Mosq. Syst. 24*: 85-101.

Le Pont, F., Caillard, T., Tibayrenec, M., Dujardin, J.P. and **Desjeux, P.** (1985). Distinction par les isoenzymes entre deux especies cryptiques au sein du taxon *Psychodopygus carrerai* (Diptera, Psychodidae). *C.R. Acad Sci. 13*: 479-481.

Lehmann, T., Hawley, W.A., Kamau, L., Fontenille, D., Simard, F. and **Collins, F.H.** (1996). Genetic differentiation of *Anopheles gambiae* populations from East and West Africa: comparison of microsatellite and allozyme loci. *Heredity 77*: 192-200.

Lehmann, T., Hawley, W.A., Grebert, H. and **Collins, F.H.** (1998). The effective population size of *Anopheles gambiae* in Kenya: implications for population structure. *Mol. Biol. Evol. 15*: 264-276.

Lerdthusnee, K. and **Chareonviriyaphap, T.** (1999). Comparison of isoenzyme patterns of *Aedes aegypti* populations collected from pre- and post-*Bacillus thuringiensis israelensis* treatment sites in Thailand. *J. Am. Mosq. Control Assoc. 15*: 48-52.

Lewontin, R.C. and **Krakauer, J.** (1973). Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. *Genetics 74*: 175-195.

Mitchell, S.E., Narang, S.K., Goldenthal, M., Cockburn, A.F. and **Seawight, J.A.** (1992). Mitochondrial and ribosomal DNA variation among members of the *Anopheles quadrimaculatus* species complex. *Genome 35*: 939-950.

Moncada-Pérez, A. and **Conn, J.** (1992). The larval polytene chromosomes of *Anopheles aquasalis* (Diptera: Culicidae) from Venezuela. *Genome 35*: 327-331.

Moran, P.A.P. (1950). Notes on continuous stochastic phenomena. *Biometrika 37*: 17-23.

Morrison, A.C., Ferro, C., Morales, A., Tesh, R.B. and **Wilson, M.** (1993). Dispersal of the sand fly

Lutzomyia longipalpis (Diptera: Psychodidae) at an endemic focus of visceral leishmaniasis in Colombia. *J. Med. Entomol. 30*: 427-435.

Mukhopadhyay, J., Rangel, E., Ghosh, K. and **Munstermann, L.** (1997). Patterns of genetic variability in colonized strains of *Lutzomyia longipapis* (Diptera: Psychodidae) and its consequences. *Am. J. Trop. Med. Hyg. 57*: 216-221.

Munstermann, L.E. (1988). Biochemical systematics of nine Nearctic *Aedes* mosquitoes (subgenus *Ochlerotatus*, *annulipes* group B). In: *Biosystematics of Haematophagus Insects*. *The Systematics Association* (Service, M.W., ed.). Claredon, Oxford, UK, pp. 134-147.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA 70*: 3321-3323.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics 89*: 583-590.

Nevo, E. (1973). Test of selection and neutrality in natural populations. *Nature 244*: 573-575.

Oden, N.L. (1984). Assessing the significance of a spatial correlogram. *Geogr. Anal. 16*: 1-16.

PAHO - Panamerican Sanitary Bureau (1972). *Venezuelan Encephalitis 1972*. Scientific Publication No. 243. Washington DC, USA, pp. 157-161.

Parkash, R. and **Yadav, J.P.** (1993). Geographical clinal variation at seven esterase-coding loci in Indian populations of *Zaprionus indianus*. *Hereditas 119*: 161-170.

Paupy, C., Vazeille-Falcoz, M., Mousson, L., Rodhain, F. and **Failloux, A.B.** (2000). *Aedes aegypti* in Tahiti and Moorea (French Polynesia): isoenzyme differentiation in the mosquito population according to human population density. *Am. J. Trop. Med. Hyg. 62*: 217-224.

Perera, O.P., Mitchell, S.E., Cockburn, A.F. and **Seawright, J.A.** (1995). Variation in mitochondrial and ribosomal DNA of *Anopheles quadrimaculatus* species A (Diptera: Culicidae) across a wide geographic range. *Ann. Entomol. Soc. Am. 88*: 836-845.

Robertson, A. and **Hill, W.G.** (1984). Deviations from Hardy-Weinberg proportions: sampling variances and use in estimation of inbreeding coefficients. *Genetics 107*: 703-718.

Rogers, J.S. (1972). Measures of genetic similarity and genetic distance. *Stud. Genet. 7*: 145-153.

Ruiz-Garcia, M. (1994a). Genetic profiles from coat genes of natural Balearic cat populations: An eastern Mediterranean and North-African origin. *Genet. Sel. Evol. 26*: 39-64.

Ruiz-Garcia, M. (1994b). Genetic structure of Marseilles cats: Is there really a strong founder effect? *Genet. Sel. Evol. 26*: 317-331.

Ruiz-Garcia, M. (1997). Genetic relationships among some new cat populations sampled in Europe: a spatial autocorrelation analysis. *J. Genet. 76*: 1-24.

Ruiz-Garcia, M. (2000). Genetic microstructure in two Spanish cat populations. II: Gametic disequilibrium and spatial autocorrelation. *Genes Genet. Syst. 75*: 281-292.

Ruiz-Garcia, M. and **Alvarez, D.** (2000). Genetic microstructure in two Spanish cat populations. I: genic diversity, gene flow and selection. *Genes Genet. Syst. 75*: 269-280.

Ruiz-Garcia, M. and **Jordana, J.** (1997). Spatial genetic structure of the "Gos d'Atura" dog breed in Catalonia (Spain). *Braz. J. Genet. 20*: 225-236.

Ruiz-Garcia, M. and **Jordana, J.** (2000). Spatial structure and gene flow from biochemical markers in the "Pyrenean Brown" breed, a rare cattle race in Catalonia (Spain). *Biochem. Genet. 38*: 341-352.

Saitou, N. and **Nei, M.** (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol. 4*: 406-425.

Slatkin, M. (1985). Rare alleles as indicators of gene flow. *Evolution 39*: 53-65.

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution 47*: 264-279.

Sneath, P.H.A. and **Sokal, R.R.** (1973). *Numerical Taxonomy*. Freeman, San Francisco, CA, USA.

Sokal, R.R. and **Oden, N.L.** (1978a). Spatial autocorrelation in biology. 1. Methodology. *Biol. J. Linn. Soc 10*: 199-228.

Sokal, R.R. and **Oden, N.L.** (1978b). Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. *Biol. J. Linn. Soc. 10*: 229-249.

Sokal, R.R., Smouse, P.E. and **Neel, J.V.** (1986). The genetic structure of a tribal population, the Yanomamas Indians. XV. Patterns inferred by autocorrelation analysis. *Genetics 114*: 259-287.

Sokal, R.R., Oden, N.L. and **Baker, J.S.F.** (1987). Spatial structure in *Drosophila buzzatii* populations: simple and directional spatial autocorrelation. *Am. Nat. 129*: 122-142.

Sokal, R.R., Harding, R.M. and **Oden, N.L.** (1989). Spatial patterns of human gene frequencies in Europe. *Am. J. Phys. Anthropol. 80*: 267-294.

Spuhler, J.P. (1972). Genetic, linguistic, and geographical distances in native North America. In: *The Assessment of Population Affinities in Man* (Wiener, J.S. and Huizinga, J., eds.). Oxford University

Press, Oxford, USA.
Tabachnick, W.J. and **Black IV, W.C.** (1995). Making a case for molecular population genetic studies of arthropod vectors. *Parasitol. Today 11*: 27-30.
Templeton, A.R. (1980). The theory of speciation via the founder principle. *Genetics 94*: 1011-1038.
Waring, G.L., Abrahamson, W.G. and **Howard, D.J.** (1990). Genetic differentiation among host-associated populations of the gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). *Evolution 44*: 1648-1655.
Watada, M., Tobari, Y.N. and **Ohba, S.** (1986). Genetic differentiation in Japanese populations of *D. simulans* and *D. melanogaster*. I. Allozyme polymorphisms. *Jpn. J. Genet. 61*: 253-269.
Wilkerson, R.C., Parsons, T.J., Klein, T.A., Gaffigan, T.V., Bergo, E. and **Consolim, J.** (1995). Diagnosis by RAPD-PCR of four cryptic species related to *Anopheles* (*Nyssorhynchus*) *albitarsis* from Paraguay, Argentina and Brazil (Diptera: Culiciade). *J. Med. Entomol. 32*: 697-704.
Wood, J.W. (1978). Population structure and genetic heterogeneity in the Upper Markham Valley of New Guinea. *Am. J. Phys. Antrop. 48*: 463-470.
Woods, P.E. and **Guttman, S.I.** (1987). Genetic variation in *Neodiprion* (Hymenoptera: Symphyta: Diprionidae) sawflies and a comment on low levels of genetic diversity within the Hymenoptera. *Ann. Entomol. Soc. Am. 80*: 590-599.
Wright, S. (1951). The genetical structure of populations. *Ann. Eugen. 15*: 323-354.
Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution 19*: 395-420.