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Distribution of Phlebotomine Sand Fly Genotypes (*Lutzomyia shannoni*, Diptera: Psychodidae) Across a Highly Heterogeneous Landscape

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ABSTRACT Genetic variability of eight Colombian field populations and two laboratory colonies of a tropical forest sand fly, *Lutzomyia shannoni* Dyar, was assessed by comparing allozyme frequencies at 20 enzyme loci. Substantial genetic variability was noted in all strains, with mean heterozygosities of 13–21% and alleles per locus of 2.0–2.8. Four loci were monomorphic. Six populations in north and central Colombia showed close genetic similarity (Nei's distances, 0.01–0.09), despite mountainous environment, discontinuous forest habitat, and elevation differences from 125 to 1,220 m. Two samples representing the Orinoco (near Villavicencio) and Amazon (near Leticia) river basins were similar (Nei's distance, 0.08) but diverged substantially from the central six samples (Nei's distances, 0.26–0.40). Although the range of *L. shannoni* extends from the southeastern United States to northern Argentina, three genetically distinct, geographically discrete, groups were discerned by the current analysis: Orinoco-Amazon river basins, north-central Colombia, and eastern United States.

KEY WORDS *Lutzomyia shannoni*, phlebotomine sand flies, species distribution and ecology, isozyme analysis, population genetics, Colombia

Lutzomyia shannoni DYAR, a phlebotomine sand fly, is distributed from the southeastern United States to Argentina (Young and Duncan 1994). It is a small fly, weighing from 0.4 mg (males) to 0.6 mg (females) (L.E.M., unpublished data). The adults are generally found in association with other sand fly species within the crevasses of buttress roots of very large tropical forest trees (Memmott 1991, 1992). Phlebotomine sand flies, particularly the *Lutzomyia* (350+ species), are not strong fliers and tend to remain associated with their emergence sites, typically not dispersing more than a half kilometer (Alexander 1987, Morrison et al. 1993).

The requirement of *L. shannoni* for blood to develop its eggs hints at its potential as a vector of leishmaniasis, a protozoan disease affecting humans throughout tropical America. Laboratory studies indicate that at least three species of *Leishmania* parasites, *Leishmania mexicana* Biagi, *L. panamensis* Lainson & Shaw, and *L. chagasi* Cunha & Muniz, can develop in *Lutzomyia shannoni* (Ferro et al. 1998). In addition, *L. shannoni* is a demonstrated vector of vesicular stomatitis in the United States, a viral disease that is infective to feral and domestic mammals (Comer et al. 1991).

The population structure of *L. shannoni* is of interest from several perspectives. First, its broad, but disjunct, distribution throughout the Americas predict genetic divergences that correspond to the degree of isolation occurring as a consequence of geographic change. Second, the tropical forests of the Americas have become fragmented, often with only small patches remaining. This provides an opportunity to examine whether insects of low vagility have a reduced genetic variability (due to genetic drift) within a patch and the degree of differentiation among patches. Third, due to difficulties of access to habitat and procurement of numerically adequate samples, no other tropical forest sand fly has been characterized for its population genetic structure, although recently, isolated populations in the mountains of southern Colombia have been compared by isoenzyme phenotypes (Cárdenas et al. 2001).

Materials and Methods

Samples. Field samples of *L. shannoni* were aspirated from the crevasses of buttress roots of forest trees or from the surface of a Shannon trap (Service 1976) in crepuscular collections. Flies were frozen immediately in liquid nitrogen at the field site, trans-

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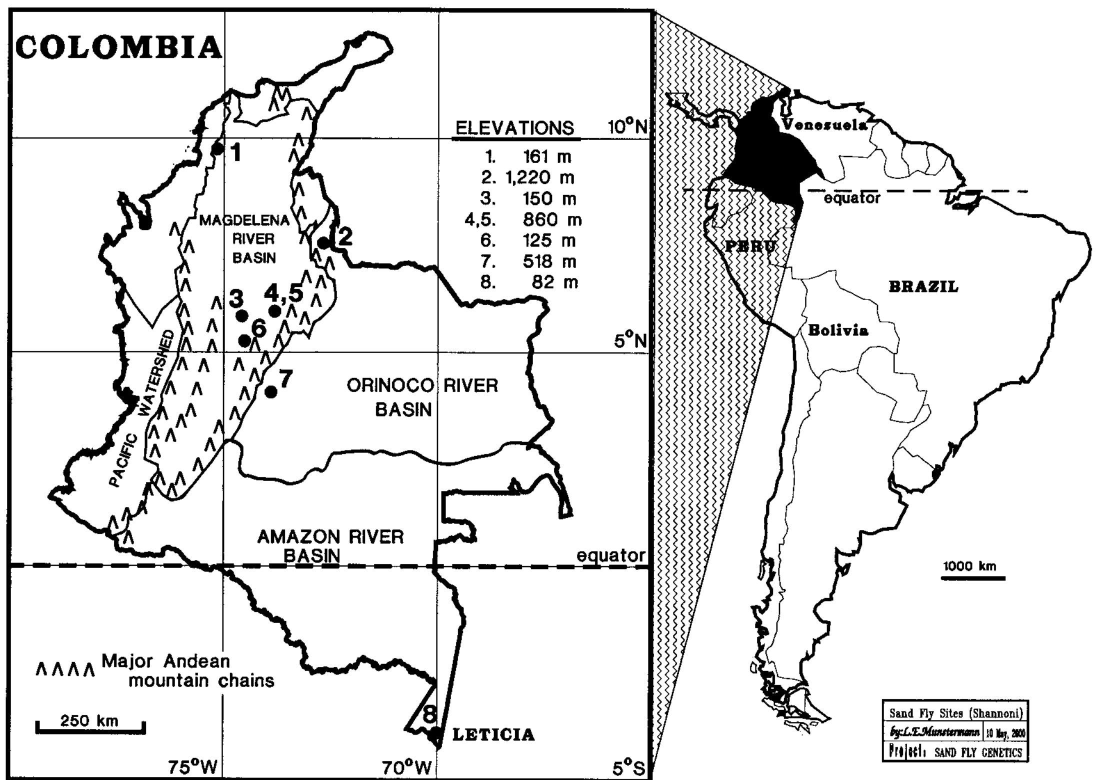

Fig. 1. Sites of collection of eight field-collected *Lutzomyia shannoni* populations in Colombia, analyzed for genetic variability. (1) Carmen. (2) Caparapí. (3) Chinácota. (4) Puerto Boyacá. (5) La Soledad. (6) Peñas Blancas. (7) Guamal. (8) Leticia.

ported to the Yale laboratory and stored at −70°C. Collection localities were distributed among three of the major river systems in Colombia as shown in Fig. 1; additional information for each locality is provided in Table 1. Morphological representatives from each field site are held in the reference collection at the Instituto Nacional de Salud, Bogotá, Colombia; voucher specimens of each sex from each site were preserved in ethanol and deposited at the Yale Peabody Museum of Natural History ($\Sigma n = 56$, specimen nos. YPM-ent 149494–149549). The two most closely related species in the Shannoni group, *Lutzomyia dendrophila* (Mangabeira) and *L. abonnenci* (Floch & Chassignet), were found in sympatry with *L. shannoni* at several locations, but were distinguished clearly from *L. shannoni* by unique electrophoretic signatures (L.E.M., unpublished data).

Three criteria drove the site selection. First, the study was restricted to a relatively small subsection of its entire range, confined within Colombia; the U.S. colony served as an external comparison. Second, landscape heterogeneity permitted site selection with varying degrees of isolation—by distance, by river basin features, by high mountains, by patchy forest habitat. Finally, the political landscape restricted collection activity in many areas, particularly in the southeast and southwest, along the western coast and in some northern sectors.

The two laboratory strains of *L. shannoni*, Bolívar and Georgia, were maintained at the insectary facility in the Department of Epidemiology and Public Health, Yale University for 3 and 2 yr, respectively, through an average of seven generations per year. These strains provided data complementary to the field samples and served as additional reference standards on each gel. The Bolívar colony was established from a large collection of adults aspirated from buttress roots of large trees (C.F., unpublished data); the Georgia colony was established from adults captured by J. A. Comer with tree hole emergence traps (Comer and Corn 1991).

Isozyme Electrophoresis. Each specimen was examined at 20 enzyme loci for isoenzyme phenotypes separated on polyacrylamide gels. Preparation of each specimen, buffer systems and gel protocols have been elaborated elsewhere (Black and Munstermann 1996, Munstermann et al. 1998). Gels were stained histochemically following Manchenko (1994). The enzyme locus abbreviation and the number assigned by the International Union of Biochemistry for each enzyme are listed as follows: adenylate kinase (*Ak*, 2.7.4.3); arginine kinase (*Ark*, 2.7.3.3); diaphorase (*Dia-1, Dia-2, Dia-3*, 1.8.1.4); esterase (*Est-1*, 3.1.1.–); fumarate hydratase (*Fum*, 4.2.1.2); glycerol 3-phosphate dehydrogenase (*Gpd*, 1.1.1.8); glucosephosphate isomerase (*Gpi*, 5.3.1.9); hexokinase (*Hk*, 2.7.1.1); iso-

Table 1. Sources of *Lutzomyia shannoni* that were compared by allozyme electrophoresis

Strain name	State	Municipality	Code[a]	Altitude, m	Latitude	Longitude	Sample
			Field samples				
Carmen	Bolívar	El Carmen de Bolívar	COBOCB-02	161	09° 43′ 20″ N	75° 07′ 59″ W	69
Caparrapí	Cundinamarca	Caparrapí	COCUCA-04	125	05° 20′ 49″ N	74° 29′ 45″ W	391
Chinácota	Norte de Santander	Chinácota	CONSCC-01	1,220	07° 36′ 35″ N	72° 36′ 14″ W	57
Guamal	Metá	Guamal (Villavicencio)	COMEGU-03	518	03° 52′ 48″ N	73° 45′ 56″ W	156
La Soledad	Santander	Landázuri	COSALA-04	800	06° 13′ 26″ N	73° 48′ 46″ W	235
Peñas Blancas	Santander	Landázuri	COSALA-09	800	06° 13′ –″ N	73° 49′ –″ W	33
Leticia	Amazonas	Leticia	COAMLE-02	82	04° 12′ 55″ N	69° 56′ 26″ W	48
Puerto Boyacá	Boyacá	Puerto Boyacá	COBOPU-01	150	05° 58′ 41″ N	74° 35′ 23″ W	160
			Colony strains				
BOLÍVAR	Bolívar	El Carmen de Bolívar	—	161	09° 43′ 20″ N	75° 07′ 59″ W	Many
GEORGIA	Georgia, USA	Ossabaw Island	—	2	31° 50′ –″ N	81° 06′ –″ W	Many

All samples were collected in Colombia, excepting the GEORGIA strain.
[a] Voucher identification and collection site code.

citrate dehydrogenase (*Idh-2,* 1.1.1.42); iditol dehydrogenase (*Idd,* 1.1.1.14); malate dehydrogenase (*Mdh-1, Mdh-2,* 1.1.1.37); 'malic' enzyme (*Me,* 1.1.1.40); mannose-6-phosphate isomerase (*Mpi,* 5.3.1.8.); phosphoglucomutase (*Pgm,* 5.4.2.2) and trehalase (*Tre-1, Tre-2, Tre-3,* 3.2.1.28).

Data Analysis. Alleles were given a numerical designation based on the distance between the origin of the gel and the leading edge of the electrophoretic band in question. *Aedes aegypti* (L.), ROCK strain, was used as a reference standard. Genotypes of each enzyme in each specimen were organized for analysis by BIOSYS-1 program (Swofford and Selander 1981, with modifications by W. C. Black IV, unpublished data) to obtain average heterozygosities, genetic distance coefficients, and dendrograms of genetic relationships. Minimum migration rates among populations were based on Wright's F_{ST} values and calculated by $F_{ST} = 1/(1+4Nm)$, where Nm is the absolute number of migrants per generation (Tabachnick and Black 1996). For loci such as *Pgm, Dia, Idd* and *Mpi,* where enzyme titer was occasionally too low to be detected visually, gel bands were resolved by computer photo-enhancement (Mukhopadhyay et al. 1997).

Results

Table 2 lists allele frequencies for each polymorphic enzyme for laboratory and field samples. The *Gpi* and *Mpi* loci were highly variable with 13 alleles per locus overall distributed among different populations. For example, two very rapidly migrating alleles of *Gpi* ($Gpi^{0.81}$ and $Gpi^{0.76}$) were restricted to two sites at the municipality of Landázuri (La Soledad and Peñas Blancas, Table 2). Four loci were monomorphic; neither sex-specific alleles or diagnostic loci were observed in any of the colonies or field samples.

For one set of five polymorphic loci (*Ak, Dia-3, Idh-2, Me* and *Mdh-1*), a single allele was the "most common allele" among all of the Colombian samples (Table 2). A second set of five loci (*Dia-2, Gpd, Hk, Mdh-2* and *Tre-2*) had the same "most common allele" in six field samples originating north and west of the eastern edge of the Andes Mountains (Fig. 1; Table 2). The two samples representing the Orinoco (Guamal) and Amazon River (Leticia) basins had different "most common" alleles; furthermore, these two samples possessed the same allele of *Fum, Gpd,* and *Hk* loci not found in the other samples (Table 2; Fig. 1). However, the major alleles $Dia\text{-}2^{1.03}$, $Mdh\text{-}2^{0.62}$, and $Pgm^{1.23}$ in Guamal also were relatively frequent in the six north central samples. $Hk^{0.88}$ was the only allele present (100%) in the Georgia laboratory colony commonly found in the Guamal (88%) and Leticia (99%) populations, but was relatively uncommon (1.2–6.4%) in the other six samples.

Table 3 shows values for three genetic variables for each sample—heterozygosity, alleles per locus, and number of polymorphic loci. The average heterozygosity values for the field samples and laboratory strains were high but varied considerably (0.133–0.213). The mean number of alleles also varied among populations (2.0–2.8).

In the two laboratory strains, three loci deviated significantly from the Hardy–Weinberg equilibrium (Table 2). In the field strains, six loci were significantly deviant from the Hardy–Weinberg equilibrium (Table 2). The highly polymorphic loci of *Gpi* and *Mpi* were the two most frequently in disequilibrium.

Table 4 summarizes the statistical deviations from Hardy–Weinberg equilibrium for each of the 16 polymorphic loci in the form of Wright's *F*-statistics. For the enzymes *Mpi, Hk, Pgm,* and *Fum,* the F_{IS} (intra population deviation) was very high and positive, reflecting a deficiency in heterozygotes. For *Mpi,* this is probably a consequence of an insufficient sample size. The high positive value for the inter-population deviation, F_{ST}, reflected genetic divergence among populations. The minimum number of migrants, Nm, was 0.4 across all strains (based on a mean F_{ST} of 0.37) and indicated the virtual absence of migration among these populations. When the six closely related populations from north Central Colombia were considered independently, the Nm value was higher (1.3 with F_{ST} 0.161), indicating some degree of gene exchange.

Table 2. Allele frequencies of 20 enzyme loci in strains of *Lutzomyia shannoni*

Locus (R_f)[a]	Laboratory strains		Field populations							
	GEORGIA	BOLÍVAR	Carmen	Caparrapí	Chiná-cota	Puerto Boyacá	La Soledad	Peñas Blancas	Guamal	Leticia
Ak										
1.16	**0.975**	**1.000**	**0.923**	***0.940***	**0.966**	**0.980**	**0.985**	**0.965**	**1.000**	**1.000**
1.30	0	0	0.026	*0.020*	0.034	0	0	0.023	0	0
1.02	0.025	0	0	0	0	0	0	0	0	0
0.94	0	0	0.051	*0.040*	0	0.020	0.015	0.012	0	0
n	59	48	39	50	29	25	33	43	43	34
Dia-2										
1.13	0.125	*0.056*	0.026	0.010	0.017	0.034	0	0.014	0.278	**0.706**
1.03	**0.761**	***0.833***	**0.962**	**0.980**	**0.931**	**0.897**	**0.803**	**0.958**	**0.722**	0.294
0.93	0.114	*0.111*	0.013	0.010	0.052	0.069	0.197	0.028	0	0
n	44	36	39	50	29	29	33	36	36	34
Dia-3										
1.13	0.011	0.014	0.026	0	0	0.034	0	0.014	0	0.015
1.04	**0.989**	**0.986**	**0.974**	**0.953**	**0.966**	**0.948**	**1.000**	**0.986**	**0.792**	**0.985**
0.88	0	0	0	0.047	0.034	0.017	0	0	0.208	0
n	44	36	39	32	29	34	33	36	36	34
Fum										
1.62	0.026	**0.581**	0.526	0.256	**0.554**	0.173	*0.333*	0.471	0	0
1.45	**0.965**	0.419	0.474	**0.744**	0.446	**0.827**	***0.667***	**0.529**	0.444	0.387
1.05	0.009	0	0	0	0	0	0	0	**0.556**	**0.532**
0.85	0	0	0	0	0	0	0	0	0	0.081
n	57	37	39	41	28	26	30	35	36	31
Gpd										
1.65	0	0.010	0.013	0	0.017	0	0	0	**0.944**	**0.963**
1.44	**1.000**	**0.979**	**0.987**	**0.961**	**0.983**	**1.000**	**0.985**	**0.926**	0.056	0.037
1.19	0	0.010	0	0.039	0	0	0.015	0.074	0	0
n	66	48	39	51	29	24	33	34	36	31
Gpi										
0.81	0	0	0	0	0	0	0.212	0	0	0
0.76	0	0	0	0	0	0	0	0.023	0	0
0.69	0.008	0	0	0	0	0	0	0	0	0
0.66	0	0	0	0	0	0	0	0.012	0.081	0.015
0.64	**0.985**	0	0	*0.050*	0	0.051	0.015	0.023	0.035	0.044
0.57	0	0	0	*0.190*	0	0	0	0.081	**0.721**	0.221
0.55	0.008	0.375	***0.737***	*0.090*	0.121	0.218	0.076	0.116	0.012	0.044
0.50	0	0	0	0	0	0.026	0	0	0	0
0.48	0	0	0	0	0.017	0.013	0	0	0	**0.353**
0.44	0	**0.594**	*0.263*	***0.480***	**0.845**	**0.667**	**0.576**	**0.663**	0.070	0.132
0.36	0	0.031	0	*0.130*	0	0	0.121	0.081	0.058	0.132
0.33	0	0	0	*0.050*	0.017	0.026	0	0	0.023	0.059
0.29	0	0	0	*0.010*	0	0	0	0	0	0
n	65	48	38	50	29	39	33	43	43	34
Hk										
0.88	**1.000**	0.022	0.064	0.012	0.017	0.026	0.045	0.014	**0.986**	**0.882**
0.83	0	**0.978**	**0.923**	**0.988**	**0.983**	**0.974**	**0.955**	**0.972**	0.014	0.118
1.05	0	0	0.013	0	0	0	0	0.014	0	0
n	67	37	39	41	29	39	33	36	36	34
Idh-2										
1.71	0.032	0	0	0	0	0	0	0	0	0
1.45	0	0	0	0	0	0	0	0	0	0
1.25	0.024	0	0	0.012	0.017	0.013	0.015	0	0	0.015
1.12	**0.944**	**0.924**	**0.987**	**0.988**	**0.983**	**0.936**	**0.970**	**1.000**	**1.000**	**0.985**
1.00	0	0.076	0.013	0	0	0.013	0.015	0	0	0
0.83	0	0	0	0	0	0.026	0	0	0	0
n	63	46	39	41	29	39	33	36	36	34
Idd										
1.60	*0.027*	0	0	0.013	0	0	0	0.015	0	0
1.33	*0.082*	0.270	0.020	0.145	0.017	0.192	**0.531**	0.273	0	0.071
1.09	***0.891***	**0.676**	**0.940**	**0.829**	**0.983**	**0.808**	0.469	**0.667**	**1.000**	**0.875**
0.91	0	0.054	0.040	0.013	0	0	0	0.045	0	0.054
n	55	37	25	38	29	13	16	33	35	28
Me										
1.07	0.008	0.219	0.231	0	0	0.038	*0.136*	0	0	0.118
1.03	0	0	0	0	0.017	0	0	0	0.035	0
1.00	**0.902**	**0.781**	**0.769**	**0.910**	**0.810**	**0.910**	***0.864***	**0.881**	**0.953**	**0.838**
0.96	0.061	0	0	0.090	0.069	0.026	0	0.107	0.012	0.044
0.92	0.030	0	0	0	0.103	0.026	0	0.012	0	0
n	66	48	39	50	29	39	33	42	43	34

Table 2. Continued

Locus (R_f)[a]	Laboratory strains		Field populations							
	GEORGIA	BOLÍVAR	Carmen	Caparrapí	Chiná-cota	Puerto Boyacá	La Soledad	Peñas Blancas	Guamal	Leticia
Mdh-1										
0.19	0	0	0	0	0.052	0	0	0.015	0	0
0.06	0	**1.000**	**1.000**	**1.000**	**0.948**	**1.000**	**1.000**	**0.985**	**1.000**	**1.000**
null	**1.000**	0	0	0	0	0	0	0	0	0
n	67	29	39	41	29	39	34	36	36	34
Mdh-2										
1.00	0	0	0	0	0	0	0	0	0	0.015
0.81	0	0	0.026	0	0	0.026	0	0	0	0.044
0.75	0.008	0	0	0.037	0	0.064	0	0.061	0.056	0
0.62	**0.992**	**1.000**	**0.974**	**0.963**	**1.000**	**0.897**	**1.000**	**0.939**	**0.764**	0.397
0.52	0	0	0	0	0	0.013	0	0	0.167	**0.515**
0.43	0	0	0	0	0	0	0	0	0.014	0.029
n	63	40	39	41	29	39	33	33	36	34
Mpi										
1.42	0	0.014	0	0	0.188	0	0	0	0	0
1.31	0	0.014	*0.071*	0	**0.313**	0	0	*0.033*	0	*0.026*
1.22	0	0.056	*0*	*0.085*	0.094	0	0.034	*0.076*	0.011	0.053
1.15	*0.027*	0.125	*0.200*	*0.096*	0.281	*0.333*	0.241	*0.196*	*0.087*	*0.211*
1.05	*0.064*	0.139	*0.200*	*0.266*	0.125	*0.333*	**0.276**	*0.196*	***0.359***	*0.132*
0.97	***0.309***	**0.347**	*0.214*	*0.234*	0	*0.167*	0.121	*0.196*	*0.130*	*0.158*
0.92	*0.136*	0.111	*0.214*	*0.170*	0	*0.167*	0.172	*0.196*	*0.120*	**0.263**
0.87	*0.282*	0.194	*0.100*	*0.128*	0	0	0.155	*0.098*	*0.293*	*0.105*
0.75	*0.145*	0	0	*0.021*	0	0	0	*0.011*	0	*0.026*
0.65	*0.036*	0	0	0	0	0	0	0	0	*0.026*
n	55	36	35	47	16	6	29	46	46	19
Pgm										
1.60	0.017	0	0	*0*	0	0.014	0	0	0	0
1.50	0.172	0.019	0	*0*	0.017	0.014	*0.083*	*0.048*	0	0
1.35	0.207	0.096	0	***0.370***	0.345	0.081	*0.333*	*0.071*	0	0
1.29	0.052	0	0	*0.019*	0	0	0	*0.024*	0	0
1.23	**0.379**	0.365	**1.000**	*0.278*	**0.534**	**0.514**	**0.500**	**0.619**	**0.524**	0.083
1.06	0.172	**0.481**	0	*0.296*	0.103	0.351	*0.083*	*0.190*	0.429	**0.833**
1.08	0	0.038	0	*0.037*	0	0.027	0	*0.048*	0.048	0.083
n	29	26	11	27	29	37	31	21	21	6
Tre-2										
1.05	**0.645**	**0.848**	**0.636**	**0.796**	**1.000**	**1.000**	**1.000**	**0.975**	0	0
1.12	0.355	0.152	0.364	0.204	0	0	0	0.025	**0.548**	0.083
0.94	0	0	0	0	0	0	0	0	0.452	**0.917**
n	38	33	11	27	29	25	6	20	21	6
Tre-3										
1.16	0	0	0	0	0	0	0	0	0.143	0
1.12	0	0	0	0	0	0	0	0	**0.619**	0.167
1.09	0.338	0.147	0.318	0.019	0	0	**1.000**	0	0.238	**0.833**
1.07	**0.662**	**0.838**	**0.682**	**0.981**	**1.000**	**1.000**	0	**1.000**	0	0
0.98	0	0.015	0	0	0	0	0	0	0	0
n	37	34	11	27	29	25	6	21	21	6

Bold numbers indicate the most common alleles. Italicized numbers indicate frequencies in a population-locus combination that deviate significantly from the Hardy–Weinberg equilibrium.

Monomorphic loci are not listed but have the following alleles—$Ark^{0.90}$, $Dia\text{-}1^{0.91}$, $Est\text{-}1^{0.90}$, $Tre\text{-}1^{1.07}$.

[a] R_f, ratio of the distance from the gel origin between a sand fly-generated electromorph and the electromorph generated by the *Aedes aegypti* reference standard.

The Rogers and Nei genetic distance (*D*) values among all the populations are shown in the matrix presented in Table 5. The genetic divergence of Leticia and Guamal from the other Colombian populations was indicated clearly, with Nei's *D* values ranging from 0.26 to 0.40. The six other Colombian populations (Carmen de Bolívar, Caparrapí, Chinácota, Peñas Blancas, La Soledad and Puerto Boyacá) were much more similar, with Nei's *D* values ranging from 0.01 to 0.09 (Table 5; Fig. 1). The same genetic relationship was presented graphically in the unweighted pair-group method with arithmetic average dendrogram based on Nei's genetic distances, where Guamal and Leticia populations showed substantial genetic divergence from the six other Colombian populations (Fig. 2). The dendrogram also illustrated the relatively high degree of divergence of the North American strain originating from Georgia.

Discussion

To date, population genetic studies of New World phlebotomine sand flies have focused largely on the sand fly *Lutzomyia longipalpis* (Lanzaro et al. 1998; Mukhopadhyay et al. 1998a, 1998b; Munstermann et al. 1998; Mutebi et al. 1998; Lampo et al. 1999; Arrivillaga

Table 3. Genetic variability in eight Colombian field samples and two laboratory colonies of *Lutzomyia shannoni* for 20 enzyme loci

Strains[a]	Mean sample size per locus	Mean no. of alleles per locus	% of loci polymorphic[b]	Mean heterozygosity	
				Direct-count	Expected by Hardy–Weinberg equilibrium
GEORGIA[a]	53.0 (±2.8)	2.5 (±0.4)	65.0	0.170 (±0.053)	0.177 (±0.057)
BOLÍVAR[a]	38.5 (±1.7)	2.3 (±0.4)	65.0	0.192 (±0.047)	0.218 (±0.056)
Carmen	31.9 (±2.5)	2.1 (±0.3)	70.0	0.192 (±0.053)	0.184 (±0.053)
Caparrapí	40.2 (±2.0)	2.6 (±0.4)	75.0	0.148 (±0.044)	0.193 (±0.059)
Chinácota	28.3 (±0.6)	2.2 (±0.3)	65.0	0.143 (±0.048)	0.150 (±0.051)
Puerto Boyacá	28.1 (±2.3)	2.5 (±0.4)	60.0	0.133 (±0.036)	0.169 (±0.051)
La Soeldad	26.4 (±2.5)	2.0 (±0.3)	55.0	0.213 (±0.067)	0.192 (±0.061)
Peñas Blancas	33.8 (±1.8)	2.8 (±0.5)	70.0	0.189 (±0.055)	0.187 (±0.057)
Guamal	34.5 (±1.8)	2.3 (±0.4)	60.0	0.182 (±0.044)	0.235 (±0.058)
Leticia	26.5 (±2.5)	2.7 (±0.5)	70.0	0.179 (±0.044)	0.243 (±0.061)

[a] Laboratory colony.
[b] A locus is considered polymorphic if more than one allele was detected.

et al. 2000). However, microhabitat associations, modes of dispersal, and population genetic structure of this human-associated fly are presumably very distinct from those of forest species such as *L. longipalpis.* The current report, together with that of Cárdenas et al. (2001), describes an initial characterization of enzyme polymorphisms among widely distributed *L. shannoni* field populations across Colombia, South America.

Twenty isozyme loci were tested, many of which were standard enzyme systems applied in previous studies with *L. longipalpis* (Mukhopadhyay et al. 1997, 1998a, 198b; Munstermann et al. 1998). Many of the loci such as *Ak, Ark, Gpd, Gpi, Hk, Idh, Me, Mpi, Pgm,* and *Tre* showed similar banding phenotypes in both species. However, in *L. longipalpis,* gel bands for the *Aat-1* and *Aat-2* loci were well separated and easily recorded. In *L. shannoni,* these two loci were not resolved, because they possessed overlapping alleles that clustered together very near the gel origin. As a consequence, *Aat-1* and *Aat-2* were not included in the *L. shannoni* analysis. Conversely, *Fum, Idd,* and the three diaphorase loci produced very distinct bands in *L. shannoni* in contrast to those of *L. longipalpis.* The loci of *Mpi* and *Gpi* were highly polymorphic and produced a large number of alleles in *L. shannoni.*

In *L. shannoni* populations from Colombia, no strictly diagnostic locus was recovered in comparing field populations. The populations most similar were field populations originating from the Magdalena River valley (Carmen) and the five populations originating from the western slope of the Andes Mountains—Caparrapí, Chinácota, La Soledad, Peñas Blancas, and Puerto Boyacá (Nei's genetic distance, 0.016–0.094). This level of differentiation without diagnostic loci is typical of conspecific, but geographically dispersed populations in other Diptera (Ayala et al. 1974, Tabachnick et al. 1979, Brust and Munstermann 1992). The six populations were separated by a maximum distance of ≈450 km (north-south) and 325 km (east-west) and differed in altitude from 125 to 1,220 m (Fig. 1); they shared similar genetic profiles, with an estimated exchange of one migrant per generation. Because of the relatively low level of differentiation among them, *L. shannoni* may be capable of dispersal over broader geographic distances than *L. longipalpis,* which appears to be poorly vagile (Alexander 1987, Morrison et al. 1993).

The genetic profiles of the Guamal population (originating from the Orinoco River basin close to the eastern foothills of the Andes Mountains at an elevation of 518 m) and the Leticia population (collected near the Amazon River at 82 m) were substantially

Table 4. Wright's *F*-statistics for 16 variable enzyme loci summarized over eight Colombian field populations of *Lutzomyia shannoni*

Locus	F_{IS}	F_{IT}	F_{ST}
Ak	0.075	0.095	0.022
Dia-2	0.051	0.304	0.266
Dia-3	−0.061	0.033	0.089
Fum	0.126	0.336	0.240
Gpd	0.059	0.855	0.846
Gpi	−0.031	0.342	0.361
Hk	0.194	0.874	0.844
Idh-2	−0.045	−0.019	0.025
Idd	−0.081	0.106	0.173
Me	0.006	0.061	0.056
Mdh-1	−0.045	0.930	0.933
Mdh-2	0.069	0.337	0.288
Mpi	0.282	0.327	0.064
Pgm	0.130	0.302	0.198
Tre-2	0.005	0.539	0.537
Tre-3	−0.047	0.603	0.621
Mean	0.088	0.423	0.367

Table 5. Nei's (above diagonal) and Roger's (below diagonal) genetic distance (*D*) among laboratory and field strains of *Lutzomyia shannoni*

Strains	GEORGIA[a]	BOLÍVAR[a]	Carmen	Caparrapí	Chinácota	Puerto Boyacá	La Soledad	Peñas Blancas	Guamal	Leticia
GEORGIA[a]	—	0.221	0.219	0.191	0.236	0.203	0.242	0.218	0.278	0.353
BOLÍVAR[a]	0.403	—	0.036	0.020	0.027	0.019	0.067	0.012	0.294	0.334
Carmen	0.405	0.179	—	0.053	0.052	0.046	0.087	0.041	0.263	0.352
Caparrapí	0.379	0.135	0.213	—	0.025	0.010	0.082	0.012	0.267	0.343
Chinácota	0.422	0.161	0.215	0.152	—	0.019	0.094	0.014	0.328	0.399
Puerto Boyacá	0.395	0.140	0.204	0.110	0.142	—	0.077	0.066	0.299	0.358
La Soledad	0.424	0.237	0.269	0.260	0.281	0.257	—	0.073	0.309	0.310
Peñas Blancas	0.403	0.113	0.190	0.112	0.120	0.094	0.248	—	0.302	0.374
Guamal	0.443	0.448	0.433	0.433	0.480	0.460	0.463	0.437	—	0.085
Leticia	0.490	0.473	0.489	0.481	0.520	0.259	0.469	0.500	0.259	—

[a] Laboratory colony.

different from all other Colombian field populations located at the western slope of the Andes and the Magdalena River basin. Genetic differences between these two groups likely reflects the presence of the Andes Mountain chain which acts as an effective geographical barrier to sand fly movement. This hypothesis also is supported by the lack of evidence for significant migration between the two sides of the Andes Mountains as estimated from the *F*-statistics ($Nm = 0.431$).

The major contributors to the distinctive genetic profile of the Guamal and Leticia populations were two loci, *Hk* and *Gpd*. $Gpd^{1.65}$ was an uncommon allele in the six other field populations, but was present at >50% frequency in the Guamal and Leticia populations. These data give evidence that *L. shannoni* is highly differentiated in portions of its range, but also indicate a high degree of genetic homogeneity, even when separated in different drainage systems, giving impetus to studies of the genetic relationships among *L. shannoni* populations in their extensions across the Amazon basin and south into the Paraguay River system. Although the above analysis emphasized the role of migration, balancing selection—acting directly on these loci or indirectly on closely linked factors—may serve to maintain genetic homogeneity in the tropical wet forest environment of the Orinoco and Amazon or the quite different dry forest environments of the western Andean slopes and the Magdalena Valley.

A peculiarity of the Georgia colony was that it was fixed for the $Hk^{0.88}$ allele, an allele frequently found (98%) in the Guamal and Leticia (88%) populations, but a minor allele (<7%) in other Colombian populations. A transect through Central American countries where *L. shannoni* is resident will be necessary to track the geographic distribution of the Georgia *Hk* allele to determine its relationship to that found in the two tropical river basins. Quite possibly, this represents a reverse or double mutation that has resulted in a genetically unrelated phenocopy. Further samples from Georgia and Central America also will shed light on the colonization effects on *L. shannoni* with respect to changes in genetic variability and to fixation of alleles by genetic drift—already described as a general phenomenon in vectors (Munstermann 1994) and a striking feature of the *L. longipalpis* colonization process (Mukhopadhyay et al. 1997). The usual indicators of colonization effects, i.e., alleles per locus and percent loci polymorphic, did not distinguish *L. shannoni* colonies from field populations (Table 2). These effects may have been mitigated by the large field collection that formed the founding colony, fewer than 15

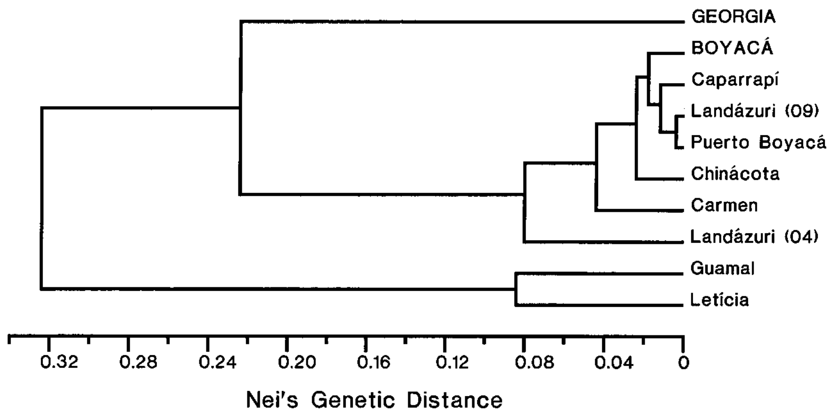

Fig. 2. Dendrogram showing Nei's genetic distance relationships among eight field-collected Colombian samples and two laboratory colonies.

or 20 generations in colony and, possibly, an innate ease in colonization that precluded a strong genetic "bottlenecking" effect.

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