Medical and Veterinary Entomology (1997) **1**, 394–400

Isozymic and metric variation in the *Lutzomyia longipalpis* complex

JEAN-PIERRE DUJARDIN,* E. M. TORREZ, F. LE PONT, D. HERVAS and D. SOSSA† Instituto de Biologia de Altura (IBBA), La Paz, Bolivia; *UMR CNRS-ORSTOM 9226, 'Génétique Moléculaire des Parasites et des Vecteurs', ORSTOM, Montpellier, France; and †Vector Control Programme, Ministry of Health, La Paz, Bolivia

Abstract. Male *Lutzomyia longipalpis* of two types from Bolivia were compared using isozyme electrophoresis and wing morphometry. One sample (*ex* Chiflonkaka Cave, alt. 2800 m at Toro Toro, Charcas Province, Potosi Department) was 'two-spot' phenotype males (i.e. tergites III and IV with paired pale patches of pheromone glands), whereas two other locality samples (Apa Apa and Imanaco, Sud Yungas Province, La Paz Department) were one-spot male phenotype (only tergite IV with paired pale patches). Multilocus enzyme electrophoresis (using ACON, aGPD, GPI, IDH, MDH, ME, 6PGD, PGM, LAP and PEPB) found no difference between samples from adjacent hen houses at Apa Apa. Nei's standard genetic distance between one-spot samples from Apa Apa and Imanaco (5 km apart, 1500 m alt.) was 0.001–0.002, whereas the two-spot males from Toro Toro (800 km away) showed a genetic distance of 0.081 from the one-spot males (Apa Apa and Imanaco). This genetic distance is commensurate with speciation, but may simply be intraspecific differentiation due to 'isolation by distance'.

For comparative wing morphometry, we included additional material of one-spot males from Bolivia (Guyabal, Sud Yungas, La Paz), Brazil, Colombia and Nicaragua. These three other country samples were assumed to be different sibling species in the complex *Lutzomyia longipalpis* (Lanzaro *et al.*, 1993). Statistics were based on univariate and multivariate analysis. The comparison between size-in and size-free canonical variate analysis (CVA) indicated that the wing morphometric divergence between one-spot and two-spot Bolivian phenotypes was not size dependent and could have taxonomic significance.

Key words. *Lutzomyia longipalpis*, sandflies, speciation, insect wings, visceral leishmaniasis, isozyme electrophoresis, morphometry, population structure, Bolivia, Brazil, Colombia, Nicaragua.

Introduction

Lutzomyia longipalpis (Lutz & Neiva) (Diptera: Psychodidae: Phlebotominae) is the principal vector of visceral leishmaniasis in Latin America, ranging from northern Mexico to Argentina. This phlebotomine sandfly preferentially inhabits dry rocky areas where it develops abundant populations in sheltered places such as caves, or peridomestic structures such as hen houses. Accordingly, it may feed on a wide variety of animals, both silvatic (e.g. *Viscacha* spp., *Cavia* spp., foxes, etc.) and domestic (dogs, hens, pigs). Variability of *Lu.longipalpis* has been revealed at morphological (Mangabeira, 1969), biochemical (Lane *et al.*, 1985; Ward, 1986; Phillips *et al.*, 1986), behavioural (Ward *et al.*, 1988) and isoenzyme (Bonnefoy *et al.*, 1986; Lanzaro *et al.*, 1993) levels. Morphological variation in males includes so-called 'one-spot' and 'two-spot' phenotypes (Mangabeira, 1969), depending on whether abdominal tergites III or III + IV have paired pale patches. The latter form occurs in Brazil, Paraguay and Bolivia (Young & Duncan, 1994). In Bolivia, 'two-spot' males were reported only in Toro Toro, Department of Potosi (Le Pont *et al.*, 1989). Le Pont (unpubl. data) also observed striking differences in the size of sympatric *Lu.longipalpis*, though no evidence for speciation was found (Bonnefoy *et al.*, 1986).

Correspondence: Dr J-P. Dujardin, IBBA, c/o Embajada de Francia, CP 717 (or ORSTOM, EP 9214, Fax 5912391416), La Paz, Bolivia.

By isoenzyme genetic studies on laboratory strains, Lanzaro *et al.* (1993) distinguished *Lu.longipalpis* from Brazil, Colombia and Costa Rica as different sibling species.

This work provides genetic data on natural populations of *Lu.longipalpis* and is the first attempt to assess genetic relationships between both spot forms in Bolivia. In addition, it evaluates variation of some morphometric characters used in taxonomy (Bermúdez *et al.*, 1991; Lebbe *et al.*, 1991). Assuming that our Nicaraguan *Lu.longipalpis* is the same species as described from Costa Rica by Lanzaro *et al.* (1993), this is also the first metric comparison between (a) the three putative species from Brazil, Colombia and Central America, (b) one-spot versus two-spot forms and (c) between locally allopatric one-spot subpopulations in Bolivia.

Material and Methods

Sandflies. Lu.longipalpis males with one pair of tergal spots were captured by aspirator from four hen houses (HI–4) at three villages (altitude 1500 m) in the province of Sud Yungas, Department of La Paz, Bolivia. H1 and H2 were in the village of Apa Apa (AA), 5 km from H3 in Imanaco (IM). H4 was in Guayabal (GU) 50 km to the northeast (Fig. 1, top). Male *Lu.longipalpis* with two pairs of tergal spots were captured by light traps in the entrance of a cave at Chiflonkaka (altitude 2800 m), Toro Toro (TT), in the province of Charcas, Department of Potosi, *c.* 800 km southeast of AA and IM (Fig. 1). Specimens were put in vials and stored in liquid nitrogen for transportation to the laboratory. Each male was confirmed as *Lu.longipalpis* by checking genitalia before processing for morphometry and electrophoresis (Table 1).

Additional samples of one-spot males of *Lu.longipalpis* used for morphometry (Table 1) were obtained from Brazil, Minas Gerais State, collected in 1956 by A. Vianna Martins (identified by Milton Moura Lima) from Colombia (CIDEIM strain) and from Nicaragua: peridomestic light trap catches collected by F.L.P. in 1986 at Somotillo and Cico Pinos villages on the Honduras border.

Isozyme electrophoresis. Cellulose acetate electrophoresis was used on individual insects according to Dujardin & Tibayrenc (1985a, b). Ten enzyme systems assayed were ACON (aconitate hydratase or aconitase, EC 4.2.1.3), aGPD (alpha-glycerophosphate dehydrogenase, EC 1.1.1.8), GPI (glucose phosphate isomerase, EC 5.3.1.9), IDH (isocitrate dehydrogenase, EC 1.1.1.42), MDH (malate dehydrogenase, EC 1.1.1.37), ME (malic enzyme, EC 1.1.1.40), 6PGDH (6-phosphogluconate dehydrogenase, EC 1.1.1.44), PGM (phosphoglucomutase, EC 5.4.2.2, formerly EC 2.7.5.1.), LAP (leucine aminopeptidase (EC 3.4.11) and PEPB (aminopeptidase B, EC 3.4.13, substrate L-leucyl-alanine).

Genetic analysis. Estimation of total (H) expected heterozygosity of putative allozymes (also known as gene diversity), their standard error (sH) (Table 2), fixation indices for subdivided populations (Table 3) as well as standard genetic distance (Ds) (Table 4) followed the formulae of Nei (1987). Comparison of gene frequencies was performed by Fisher's exact test (STATA, 1992; Hamilton, 1993).

Wing morphometry. Five micromorphometric characters (Fig. 2) were measured on the dry wings of each specimen: *alpha*, the length of R2 from its junction with R3 to the costa; *beta*, the length of R from junction of R2&R3 to junction with R4; *gamma*, the length of R from origin of R5 to origin of R2+3&R4; *delta*, the part of R1 extending beyond junction of R2 and R3; and the length of *R5* (Fig. 2). All measurements were made by the same investigator (E.M.T.) at 12.5 (ocular) ×10 (objective) magnification, except R5 needed 12.5 × 4 magnification.

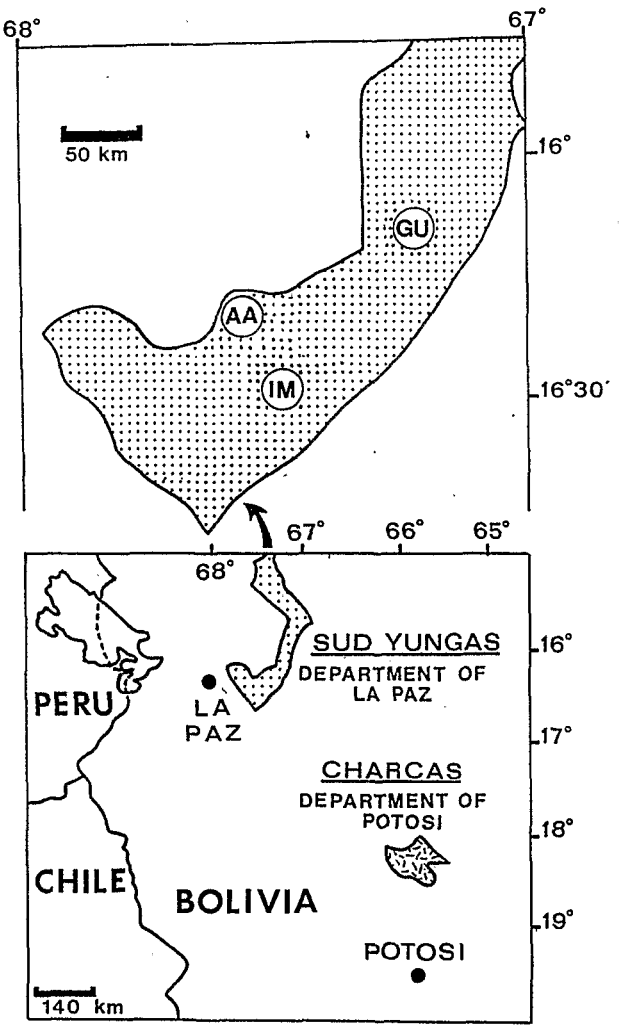


Fig. 1. Maps showing localities in Bolivia where males of *Lutzomyia longipalpis* were collected Bottom: Sud Yungas (Department of La Paz) and Charcas (Department of Potosi). Top: relative locations of three sites within Sud Yungas province: Apa Apa (AA), Imanaco (IM) and Guayabal (GU).

Numerical analysis. Since no directional asymmetry (the mean value of one side systematically larger than the mean of the other) was found, comparisons between localities were performed on wings rather than on individuals. Mean and standard error were calculated after adequate transformation of each measurement unit (Table 5). Univariate analysis used non-parametric tests (Kruskal & Wallis, 1952; STATA, 1992; Hamilton, 1993). Multivariate statistics compared all morphological characters simultaneously, except for the *delta* distance which was removed due to its excessive redundancy with *alpha*. To equalize variances

Table 1. Samples of *Lutzomyia longipalpis* males examined (number of wings in parentheses); see Materials and Methods section for details.

Sample	Code	Tergal spot phenotype	No. examined: Wing morphometry	No. examined: Electrophoresis
Bolivia, Sud Yungas	AA H1	1	—	24
	AA H2	1	42 (84)	26
	IM H3	1	14 (27)	24
	GU H4	1	19 (37)	0
Charcas	TT	2	12 (23)	29
Brazil, Minas Gerais	BR	1	3 (6)	0
Colombia, CIDEIM	CO	1	13 (26)	0
Nicaragua	NI	1	13 (26)	0
Total			116 (229)	103

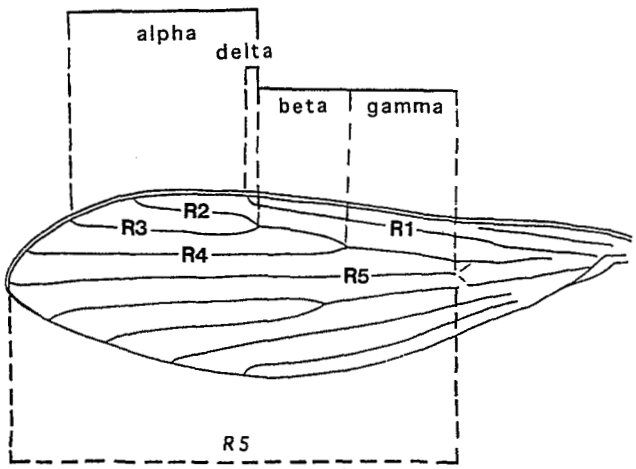


Fig. 2. Wing of male *Lutzomyia longipalpis* (dorsal aspect) showing measurements taken: for key see text on wing morphometry and Young & Duncan (1994: Fig. 17A).

among groups and variables, data were first transformed to logarithms (Yoccoz, 1993). A covariance-matrix based principal component analysis (PCA) was used to summarize total variation. The coefficients of PC-1 were positive, as well as positively and significantly correlated with the values of wing characters, indicating PC1 represents a general size factor (Table 6) (Strauss, 1985). The effect of size variation was removed by regressing each character separately on the first principal component (PC1), and performing canonical variate analysis (CVA) on residuals (size-free CVA). The significance of size-in and size-free CVA was tested by a between-groups permutations test computing the occurence of a between-inertia as high or higher than observed (software ADE 4.0; Chessel & Dolédec, 1992). Mahalanobis distance were used for constructing an UPGMA (unweighted pair group method analysis) tree, either from size-in (Fig. 3, top) or size-free CVA (Fig. 3, bottom)

Results

Isozyme electrophoresis

6PGDH was not scorable on gels; aGPD and PEPB showed variable patterns, not amenable to allelic interpretation. Six enzymes showed only one band, indicating single loci, whereas MDH exhibited two bands that segregated at separate loci (*Mdh1*

Table 2. Genetic parameters for *Lutzomyia longipalpis* samples from four Bolivian localities. Af = Allele frequency of the most frequent allele in the total sample; Moh = Mean observed heterozygosity; Meh = Mean expected heterozygosity; SE = standard error of Meh; TT = Toro Toro; IM = Imanaco, henhouse H3; AA = Apa Apa, henhouses H1 and H2.

Factor		Locality sample: TT	IM (H3)	AA (H1)	AA (H2)	Total
Af	*Gpi*	1	1	0.89	0.92	0.95
	Pgm	0.19	1	1	1	0.77
	Mdh1	1	0.87	0.94	0.9	0.93
Moh		0.028	0.023	0.031	0.033	0.029
Meh		0.035	0.025	0.035	0.037	0.064
(SE)		(0.035)	(0.025)	(0.024)	(0.025)	(0.040)

Table 3. F-statistics at three enzyme loci of *Lutzomyia longipalpis* in Bolivia. Fis and Fit vary between −1 and +1, representing Hardy-Weinberg disequilibrium measures within groups and in the total sample, respectively. Fst ranges between 0 and 1, representing the level of genetic differentiation between groups. χ^2 were calculated according to the following formulae: χ^2Fis = n.Fis^2 and χ^2Fit = n.Fit^2 with one degree of freedom (df), χ^2Fst = 2.n.Fst with 3 df. Significance of indices indicated by asterisks: *P < 0.05,**P < 0.02, ***P < 0.001.

Locus	Fis	Fit	Fst
Gpi	−0.083	−0.010	0.0675**
Pgm	0.010	0.761**	0.759***
Mdh1	−0.091	−0.037	0.049*

and *Mdh2*), giving a total of nine scorable loci. Three loci showed apparent allelic polymorphism: *Pgm* and *Mdh1* each with three alleles, and *Gpi* with two alleles. Proportions of polymorphic loci were 11% (1/9) in Toro Toro and 22% (2/9) in the Yungas. Mean expected heterozygosity was 0.064 ± 0.040 in the total sample, comprising 0.035 ± 0.035 at TT plus 0.033 ± 0.022 at the Yungas, AA and IM (Table 2). For the three polymorphic loci, fixation indices were highly significant regarding Fst ($P <$ 0.05 to <0.001), but not for Fis (Table 3). In the two samples from AA (H1 and H2) none of the isozymes differed significantly. Between AA (H1 or H2) and IM (H3) samples, gene fre-quencies at one locus (*Gpi*) were significantly different ($P < 0.02$). All three polymorphic loci differed significantly between one-spot (AA and IM) and two-spot (TT) samples: $P < 0.02$ to <0.002 (results not shown). The standard genetic distance (Nei, 1987) between one-spot and two-spot forms of *Lu.longipalpis* was 0.081 (Table 4).

Univariate analysis of morphometric data. To test for asymmetry, mean values of each wing character were compared for wings on left and right sides. Negative results (statistics not shown) demonstrated high degrees of wing symmetry in all samples.

Largest wings (*R5* up to 1.360 mm) were found in Bolivia, while the smallest wings came from Nicaragua (*R5* 0.900 to 1.180). Samples from Colombia (the only insectary-reared material used in this study) and Brazil exhibited intermediate *R5* values. This cline was found for character *alpha* also. The largest *beta* and *gamma* but the smallest *delta* were found in Colombia (Table 5). The most variable wing character, assessed from the coefficient of variation (standard deviation divided by the mean, on log-transformed data), was *delta*: 11% overall, compared with 2.92% or less for the other morphometric wing characters (Table 5).

Multivariate analysis Three groups were distinguished after size-in (conventional) CVA (Fig. 3, top): one group represented by Colombia only (CO); a second group composed of Nicaragua (NI) and Brazil (BR), and a third group subdivided into one-spot (AA, IM, GU) and two-spot male *Lu.longipalpis* (TT) of Bolivia. The removing of size variation (Fig. 3, bottom) individualized the two-spot phenotype (TT) as an independent group, external to the one-spot Bolivian phenotypes (AA, IM, GU) now clustered with Brazil and Nicaragua (BR, NI). Colombia remained as an isolated group (CO).

Table 4. Nei's standard genetic distance (Ds) between four Bolivian locality samples of *Lutzomyia longipalpis*, computed from nine loci. TT = two-spot-males from Chiflonkaka cave, Toro Toro; compared with three samples of one-spot males from henhouses: H1 and H2 at Apa Apa (AA), H3 at Imanaco (IM). Geographic distances between localities (Table 1, Fig. 1) are 5 km between AA (H1 and H2) and IM (H3); >500 km between TT and the cluster of other sites (H1+H2 at AA, H3 at IM).

	Locality sample		
One-spot	TT two-spot	H3 (IM) one-spot	H1 (AA) one-spot
H3 (IM)	0.080		
H1 (AA)	0.081	0.002	
H2 (AA)	0.081	0.001	0.000

Table 5. Morphometric values (microns) of five wing characters (Fig. 2) in *Lutzomyia longipalpis* from seven sites. (see Table 1). Number of wings measured (*N*), minimum (Min), maximum (Max), mean (Mean) and standard deviation (SD). The coefficient of variation (logCV) was computed on log-transformed data.

Wing character (Fig. 2)	Sample site (Table 1)	*N*	Min	Max	Mean	SD	logCV
alpha	TT	23	192	408	315	56	3.41
	IM	27	304	408	371	22	1.03
	AA	84	288	448	371	35	1.60
	GU	37	280	432	362	39	1.87
	CO	26	248	352	294	31	1.84
	BR	6	240	288	267	22	1.49
	NI	26	224	304	259	21	1.47
Total		229	192	448	340	54	2.92
beta	TT	23	200	272	233	19	1.52
	IM	27	192	272	234	19	1.46
	AA	84	200	288	239	21	1.63
	GU	37	192	288	238	24	1.83
	CO	26	240	280	265	11	0.74
	BR	6	192	208	200	7	0.68
	NI	26	160	216	190	16	1.64
Total		229	160	288	234	27	2.20
gamma	TT	23	272	392	338	33	1.74
	IM	27	280	376	337	24	1.26
	AA	84	264	400	340	31	1.59
	GU	37	216	384	288	53	3.26
	CO	26	328	424	360	21	0.98
	BR	6	264	360	303	42	2.37
	NI	26	240	304	277	22	1.42
Total		229	216	424	325	43	2.43
delta	TT	23	–40	56	22	23	24.81
	IM	27	24	96	72	18	3.82
	AA	84	– 8	120	58	28	7.02
	GU	37	16	120	71	25	5.15
	CO	26	–16	80	19	24	9.33
	BR	6	8	32	21	10	3.83
	NI	26	0	56	33	14	4.81
Total		229	–40	120	50	31	11.00
R5	TT	23	1000	1360	1223	105	1.27
	IM	27	1160	1300	1233	43	0.49
	AA	84	1080	1360	1247	61	0.70
	GU	37	1060	1260	1168	54	0.66
	CO	26	1160	1320	1221	44	0.50
	BR	6	1000	1140	1047	65	0.88
	NI	26	900	1080	1002	57	0.83
Total		229	900	1360	1194	100	1.24

Discussion

Isozyme electrophoresis

A previous study of *Lutzomyia longipalpis* variation in Bolivia by Bonnefoy *et al.* (1986) was restricted to the one-spot male form in Santa Barbara, a small village of La Paz Department, where specimens showed a wide range of size. Biallelic variability

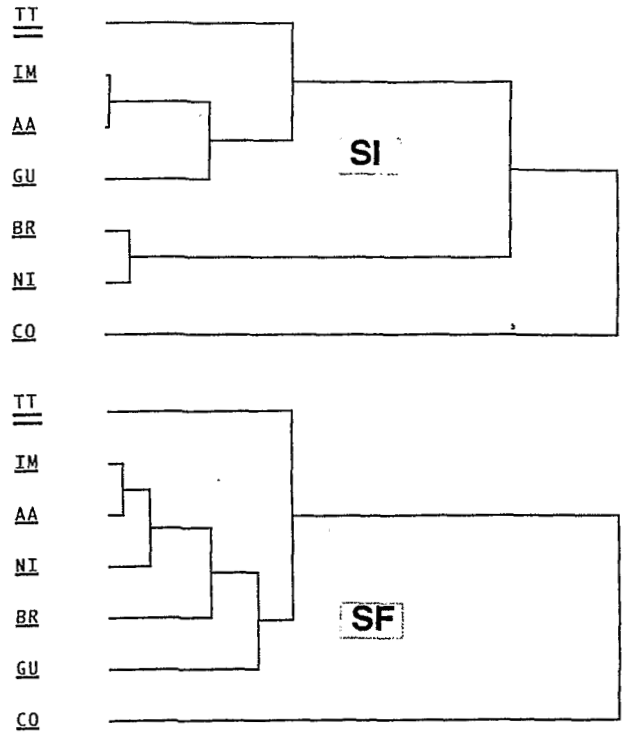


Fig. 3. UPGMA dendrograms derived from Mahalanobis distances after size-in (SI, top) and size-free (ŚF, bottom) canonical variate analysis (CVA), between seven samples of *Lutzomyia longipalpis*. Males with one pair of tergal spots from six localities: Apa Apa, Imanaco and Guayabal (AA, IM and GU), La Paz, Bolivia; Nicaragua, near Honduras border (NI); Minas Gerais, Brazil (BR) and Colombia (CO) CIDEIM insectarium strain; and males with two pairs of tergal spots from Toro Toro (TT), Potosi, Bolivia. Double and single underlining indicate two-spot and one-spot phenotypes.

was detected at two enzyme loci: *Mdh1* and *Gpi*. From wider geographic sampling in the present study, we detected an additional allele at the *Mdh1* locus plus polymorphism (three alleles) at the *Pgm* locus. We estimated mean expected heterozygosities of 0.033–0.064 for *Lu.longipalpis*, within the range of values reported previously for this taxon (Bonnefoy *et al.*, 1986; Lanzaro *et al.*, 1993), but lower than values of 0.120 reported for *Lu.carrerai* (Barretto) and *Lu.yucumensis* (Le Pont *et al.*) by Caillard *et al.* (1985), 0.098–0.211 for *L.trapidoi* F. & H. by Dujardin *et al.* (1997) or 0.082–0.171 for the *Lu.verrucarum* (Townsend) group (Kreutzer *et al.*, 1990).

Degrees of genetic difference between samples of *Lu.longipalpis* from each site were related to their geographic distances apart (Table 3). According to Nei (1987), a significant difference at one locus makes any estimate of genetic distance differ significantly from 0. Thus, the genetic distances between samples from AA (H1 + H2) and IM (H3) (Ds = 0.001–0.002, Table 4), separated by only 5 km, may well reflect true genetic differentiation. This could be attributed to apomixia between villages AA and IM, due to the limited flight range of *Lu.longipalpis*, usually <300 m (Morrison *et al.*, 1993). Samples from H1 and H2, only 50 m apart at AA, were isozymically identical (Ds = 0.000). The genetic distance of 0.081 between TT (two-spot) and AA or IM (one-spot) samples could be due to their geographic separation by >500 km or to their intrinsic reproductive divergence caused by, or at least correlated with, their different spot phenotypes.

F-statistics confirmed that spatial separation is correlated with genetic heterogeneity: Fst was sighificantly different from zero at *Gpi*, *Pgm* and *Mdh1*, indicating strong differential structuring of Bolivian *Lu.longipalpis* populations at each locality. Panmixia within each locality population was supported by Fis not differing significantly from zero (Table 3). By the criteria of Richardson *et al.* (1986), these results and their concordance with geographic distances would suggest an 'isolation by distance' model as best fitting the population pattern of *Lu.longipalpis*.

The considerable genetic distance (Ds = 0.081) between TT two-spot and the one-spot Sud Yungas (AA & IM) samples, as well as the very high value of Fst = 0.759 at the *Pgm* locus raise the question of the relative taxonomic status of these two forms of *Lu.longipalpis*. Our allopatric one- and two-spot population samples from the Bolivian Andes differ more than some sibling species of *Anopheles* mosquitoes (Bullini & Coluzzi, 1982; Lanzaro *et al.*, 1988) and *Drosophila* fruitflies (Sene & Carson, 1977). However, higher interspecific genetic distances (0.121–0.333) have been reported by Lanzaro *et al.* (1993) between members of the *Lu.longipalpis* complex, as well as between *Lu.(P.)yucumensis* and *Lu.(P.)carrerai* (Le Pont *et al.*, 1985; Caillard *et al.*, 1986) or among species of the *Lu.trapidoi* complex (Dujardin *et al.*, 1996), whereas genetic distances between conspecific populations of sandflies are only 0.004±0.004 (Lanzaro & Warburg, 1995). This highlights the need for more studies on genetic variability within and between natural populations – rather than on insectary colonies, to allow better taxonomic use of isoenzyme electrophoresis data (Tabachnick & Black, 1995).

As Nei's genetic distance cannot be used to determine species *per se*, something more objective is required to decide whether Bolivian one-spot and two-spot *Lu.longipalpis* males belong to different species. Reproductive isolation is the main biological criterion of speciation (Templeton, 1989). Accordingly, Lanzaro *et al.* (1993) recognized three apomictic species of the *Lu.longipalpis* complex from Brazil, Colombia and Costa Rica, and Ward *et al.* (1988) recognized pheromonally distinct species sympatric in Brazil. Both pheromonal types occur widely in Latin America as populations with various spot male phenotypes (see Fig. 16.3 in Ward *et al.*, 1988). Evidently the tergal spots are glands producing specific sexual pheromone (Lane *et al.*, 1985; Phillips *et al.*, 1986) serving as a precopulatory barrier separating species (Ward, 1986; Ward *et al.*, 1988). If different pheromones can be shown to prevent (i.e. fail to cause) interbreeding between our two Bolivian forms, this would explain their genetic differentiation as probable speciation.

Morphometry

Sandfly wing morphometry is being used increasingly for species characterization (Bermudez *et al.*, 1991; Lebbe *et al.*, 1991). However, morphological differences between natural populations result from both environmental and genetic differences. In *Drosophila*, three genetic loci govern wing venation (Thompson, 1975), modulated by environmental factors. Wing length variation in Diptera may arise from different larval diets (Hillesheim &

Stearns, 1991), different climates (Kitthawee *et al.*, 1992) or even larval parasitism (Siegel *et al.*, 1992). In sandflies, intraspecific variation of wing length occurs seasonally (Ogusuku *et al.*, 1993), inversely related to temperature as in mosquitoes (Le Sueur & Sharp, 1991), and proportional to altitude (Le Pont, unpubl. data), perhaps following Allen's rule (Ray, 1960).

The many environmental factors acting on size differentiation justify our attempt to remove size variation from the metric comparisons (Table 6). The statistical procedure used here, which is also a recommended way to avoid spurious results due to sampling artefacts (Dos Reis *et al.*, 1990; Yoccoz, 1993), allows the partitioning of environmental (size related differences) from evolutionary influences (Hutcheson *et al.*, 1995).

Size-in CVA showed Colombian *Lu.longipalpis* as an external group, possibly due to their origin as insectary specimens: reared sandflies have generally larger size than their natural counterparts (Lane, 1988; Klowden *et al.*, 1988; Gebre-Michael & Lane, 1993). However, size-free analysis did not modify the Colombia sample position, giving confidence that Colombian *Lu.longipalpis* could be a different species as described by Lanzaro *et al.* (1993).

Comparison between size-in and size-free CVA indicated that the separation of the Bolivian one-spot male *Lu.longipalpis* from Brazil and Nicaragua was size dependent. This could suggest that the one-spot Bolivian phenotypes are conspecific with the Brazilian (because of shape similarity), but the grouping of Nicaragua with Brazil was not expected. We assumed that the Nicaragua sample, for its geographic proximity to Costa Rica, belongs to the Costa Rican species of Lanzaro *et al.* (1993). In that case (according to the genetic distances estimated by those authors) Nicaragua should stand next to Colombia, and both of them far from Brazil. This incongruity is probably due to the fact that our material was different from the strains investigated by Lanzaro *et al.* (1993).

The two-spot male *Lu.longipalpis* of Toro Toro appeared as a consistently distinct lineage. Indeed, divergence between two-spot and one-spot Bolivian male *Lu.longipalpis*, already apparent in size-in analysis, increased after removing size variation. This, together with *c.* 0.08 genetic distance between them, could make them homologous with the pheromonally distinct sympatric sibling species first recognized in Brazil by Ward (1986). This hypothesis can be tested biochemically, by crossing experiments and by further genetic profiling of populations.

Table 6. Criterion of Strauss (1985) to interpret the first principal component (PC-1) as a general size variable after principal component analysis (PCA) involving four wing characters for male *Lutzomyia longipalpis* from Bolivia (four samples). Colombia, Brazil and Nicaragua (see Table 1). PC-1 = coefficients of the first principal component (all positive), r = correlation coefficients between the first eigenvector (PC-1) and the wing characters, all significant (***$P<0.0001$).

Character	PC-1	r
alpha	0.726	0.88***
beta	0.353	0.60***
gamma	0.433	0.63***
R5	0.401	0.93***

Acknowledgments

This work was supported by ORSTOM and the French Ministry of Foreign Affairs. We thank Dr J. Mouchet (ORSTOM, Paris) for revising this manuscript, and Dr M. Simier (Cellule de Biométrie et Statistique, ORSTOM Montpellier) for helping us in the calculation of the Mahalanobis distances. We are grateful to Dr G. Olivares (Chulumani Hospital) for his valuable field assistance, as well as to Dr N. Pereira (Director of Regional Epidemiology, La Paz) and to Dr G. Lopez (Director of the Vector Control Programme, La Paz) for helping this investigation.

References

Bermúdez, H., Dedet, J.-P., Falcao, A., Feliciangeli, D., *et al.* (1991) Proposition of a standard description for Phlebotomine sandflies. *Parassitologia*, **33**, (Suppl. 1), 127–135.

Bonnefoy, S., Barnabe, C., Tibayrenc, M., Le Pont, F., Dujardin, J.P., Desjeux, P. & Ayala, F.J. (1986) An isozymic study of *Lutzomyia longipalpis* (Diptera, Psychodidae), the vector of visceral leishmaniasis in the 'Yungas' (Bolivia). *Cahiers O.R.S.T.O.M., Série Entomologie Médicale et Parasitologie*, 24, 213–217.

Bullini, L. & Coluzzi, M. (1982) Evolutionary and taxonomic inferences of electrophoretic studies in mosquitoes *Recent Developments in the Genetics of Insect Disease Vectors* (ed. by W. Steiner, W. Tabachnick, K. Rai and S. Narang), pp. 465–482. Stipes Publishing Co., Champaign, Illinois.

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

Chessel, D. & Dolédec, S. (1992) *ADE Version 3.7: HyperCard © Stacks and Programme library for the Analysis of Environmental Data.* 9 fasc. [in French]. URA CNRS 1451, Université Lyon 1, 69622 Villeurbanne, 820pp.

Dos Reis. S.F., Pessoa, L.M. & Strauss, R.E. (1990) Application of size-free canonical discriminant analysis to studies of geographic differentiation. *Brazilian Journal of Genetics*, **13**, 509–520.

Dujardin, J.P., Le Pont, F., Cruz, M., Guilvard, E., Echeverria, R. & Tibayrenc, M. (1993) Phlébotomes, populations naturelles et génétique des populations. *Archives de l'Institut Pasteur, Tunis*, **70**, 435–442.

Dujardin, J.P., Le Pont, F., Cruz, M., Leon, R., Tarrieu, F., Guderian, R., Echeverria, R. & Tibayrenc, M. (1996) Cryptic speciation in *Lutzomyia (Nyssomyia) trapidoi* (Fairchild & Hertig) (Diptera: Psychodidae) detected by Multilocus Enzyme Electrophoresis. *American Journal of Tropical Medicine and Hygiene*, **54**, 42–45.

Dujardin, J.P. & Tibayrenc, M. (1985a) Etude de 11 enzymes et données de génétique formelle pour 19 loci enzymatiques chez *Triatoma infestans*. *Annales de la Societé Belge de Médecine Tropicale*, **65**, 271–280.

Dujardin, J.P. & Tibayrenc, M. (1985b) Etudes isoenzymatiques du vecteur principal de la maladie de Chagas: *Triatoma infestans* (Hemiptera: Reduviidae). *Annales de la Société Belge de Médecine Tropicale*, **65** (Suppl. 1), 165–169.

Gebre-Michael, T. & Lane, R.P. (1993) Distinguishing the females of *Phlebotomus* (*Synphlebotomus*) *martini* and *P.*(*S.*)*celiae* (Diptera: Phlebotomine), vectors of visceral leishmaniasis in Southern Ethiopia. *Bulletin of Entomological Research*, **83**, 353–360.

Hamilton, L.C. (1993) *Statistics with Strata®* 3. Duxbury Press. Wadsworth Publishing Co., Belmont, California. 194pp.

Hillesheim, E. & Stearns, S.C. (1991) The responses of *Drosophila Melanogaster* to artificial selection on body weight and its phenotypic

plasticity in two larval food environments. *Evolution*, **45**, 1909–1923.

Hutcheson H.J., Oliver, J.H., Houck, M.A. & Strauss, R.E. (1995) Multivariate morphometric discrimination of nymphal and adult forms of the blacklegged tick (Acari: Ixodidae), a principal vector of the agent of Lyme disease in Eastern North America. *Journal of Medical Entomology*, **32**, 827–842.

Kitthawee, S., Edman, J.D. & Upatham, E.S. (1992) Relationship between female *Anopheles dirus* (Diptera: Culicidae) body size and parity in a biting population. *Journal of Medical Entomology*, **29**, 921–926.

Klowden, M.J., Blackmer, J.L. & Chambers, G.M. (1988) Effects of larval nutrition on the host-seeking behaviour of adult *Aedes aegypti* mosquitoes. *Journal of the American Mosquito Control Association*, **4**, 73–75.

Kreutzer, R.D., Palau, M.T., Morales, A., Ferro, C., Felicianceli, D. & Young, D.G. (1990) Genetic relationships among phlebotomine sandflies (Diptera: Psychodidae) in the *verrucarum* species group. *Journal of Medical Entomology*, **27**, 1–8.

Lane, R.P. (1988) Geographic variation in Old World phlebotomine sandfiles. pp. 77–90 in Service, M.W. (ed.) *Biosystematics of Haematophagous Insects*. Clarendon Press, Oxford.

Lane, R., Phillips, A., Molyneux, D.H., Procter, G. & Ward, R.D. (1985) Chemical analysis of the abdominal glands of two forms of *Lutzomyia longipalpis*: site of a possible pheromone? *Annals of Tropical Medicine and Parasitology*, **81**, 311–317.

Lanzaro, G., Narang, S., Mitchell, S., Kaiser, P. & Seawright, J. (1988) Hybrid male sterility in crosses between field and laboratory strains of *Anopheles quadrimaculatus* (Say) (Diptera: Culicidae). *Journal of Medical Entomology*, **25**, 248–255.

Lanzaro, G.C., Ostrovska, K., Herrero, M.V., Lawyer, P.G. & Warburg, A. (1993) *Lutzomyia longipalpis* is a species complex: genetic divergence and interspecific hybrid sterility among three populations. *American Journal of Tropical Medicine and Hygiene*, **48**, 839–847.

Lanzaro, G.C. & Warburg, A. (1995) Genetic variability in phlebotomine sandflies: possible implications for leishmaniasis epidemiology. *Parasitology Today*, **11**, 151–154.

Lebbe, J., Torrez, M.E., Vignes, R. & Dedet, J.P. (1991) Morphométrie alaire et caractérisation spécifique des phlébotomes. *Parassitologia*, **33**, (Suppl. 1), 365–372.

Le Pont, F., Caillard, T., Tibayrenc, M., Dujardin, J-P. & Desjeux, P. (1985) Génétique. Distinction par les isoenzymes entre deux espèces cryptiques au sein du taxon *Psychodopygus carrerai* (Diptera, Psychodidae) *Comptes Rendus de l'Académie des Sciences de Paris*, tome 300, Série III, **13**, 479–481.

Le Pont, F., Mouchet, J. & Desjeux, P. (1989) Phlébotomes de Bolivie. VII. Répartition des deux morphotypes du phlébotome *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera, Psychodidae) dans le piémont andin de Bolivie. *Memorias ob Instituto Oswaldo Cruz*, **84**, (3), 423–426.

Le Seuer, D. & Sharp, B.L. (1991) Temperature-dependent variation in *Anopheles merus* larval head capsule width and adult wing length: implications for anopheline taxonomy. *Medical and Veterinary Entomology*, **5**, 55–62.

Mangabeira, O. (1969) Sobre a sistemática e biologia dos *Phlebotomus* do Ceará. Rev. bras. Malar. *Doenças Tropicaes*, **21**, 3–26.

Morrison, A.C., Ferro, C., Morales, A., Tesh, R.B. & Wilson, M.L. (1993) Dispersal of the sand Fly *Lutzomyia longipalpis* (Diptera: Psychodidae) at an endemic focus of visceral leishmaniasis in Colombia. *Journal of Medical Entomology*, **30**, 427–435.

Nei, M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press.

Ogusuku, E., Perez, E. & Campos, M. (1993) Nota Cientifica: Variacion estacional de las dimensiones alares de *Lutzomyia verrucarum* (Diptera: Psychodidae). *Revista Peruana de Entomología*, **34**, 55.

Phillips, A., Ward, R.D., Ryan, L., Molyneux, D.H., Lainson, R. & Shaw, J.J. (1986) Chemical analysis of compounds extracted from the tergal 'spots' of *Lutzomyia longipalpis* from Brazil. *Acta Tropica*, **43**, 271–276.

Ray, C. (1960) The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology*, **106**, 85–108.

Richardson, Adams & Baverstock, (1986) *Allozyme Electrophoresis: a Handbook for Animal Systematics and Population Studies*. Academic Press, Orlando, Florida.

Sene, F.M. & Carson, H.L. (1977) Genetic variation in the Hawaiian *Drosophila*. IV. Allozymic similarity between *D.silvestris* and *D.heteroneura* from the island of Hawaii. *Genetics*, **86**, 187–198.

Siegel, J.P., Novak, R.J. & Maddox, J.V. (1992) Effect of Ascogregarina barreti (Eugregarinida: Lecudinidae) infection on *Aedes triseriatus* (Diptera: Culicidae) in Illinois. *Journal of Medical Entomology*, **29**, 968–973.

STATA (1992) STATA Software. Computing Resource Center.

Strauss, R.E. (1985) Static allometry and variation in body form in the South American catfish genus *Corydoras* (Callichthyidae). *Systematic Zoology*, **34**, 381–396.

Tabachnick, W.J. & Black, W.C.(1995) Making a case for molecular population genetics: studies of arthropod vectors. *Parasitology Today*, **11**, 27–29.

Templeton, A.R. (1989) The meaning of species and speciation: a genetic perspective. In: *Speciation and its Consequences* (ed. by D. Otte and J. A. Endler), pp. 3–27. Sinauer, Sunderland, Massachusetts.

Thompson, J.N. (1975) Quantitative variation and gene number. *Nature*, **258**, 665–668.

Ward, R.D. (1986) Mate recognition in a sandfly (Diptera: Psychodidae). *Journal of the Royal Army Medical Corps*, **132**, 132–134.

Ward, R.D., Phillips, A., Burnet, B. & Marcondes, C.B. (1988) The *Lutzomyia longipalpis* complex: reproduction and distribution. *Biosystematics of Haematophagous Insects* (ed. by M. W. Service), pp. 257–269. Systematics Association, Special Volume No. 37.

Yoccoz, N. G. (1993) Morphométrie et analyses multidimensionnelles: Une revue des méthodes séparant taille et forme *Biométrie et Environnement* (ed. by J. D. Lebreton and B. Asselain), pp. 74–99, Masson, Paris.

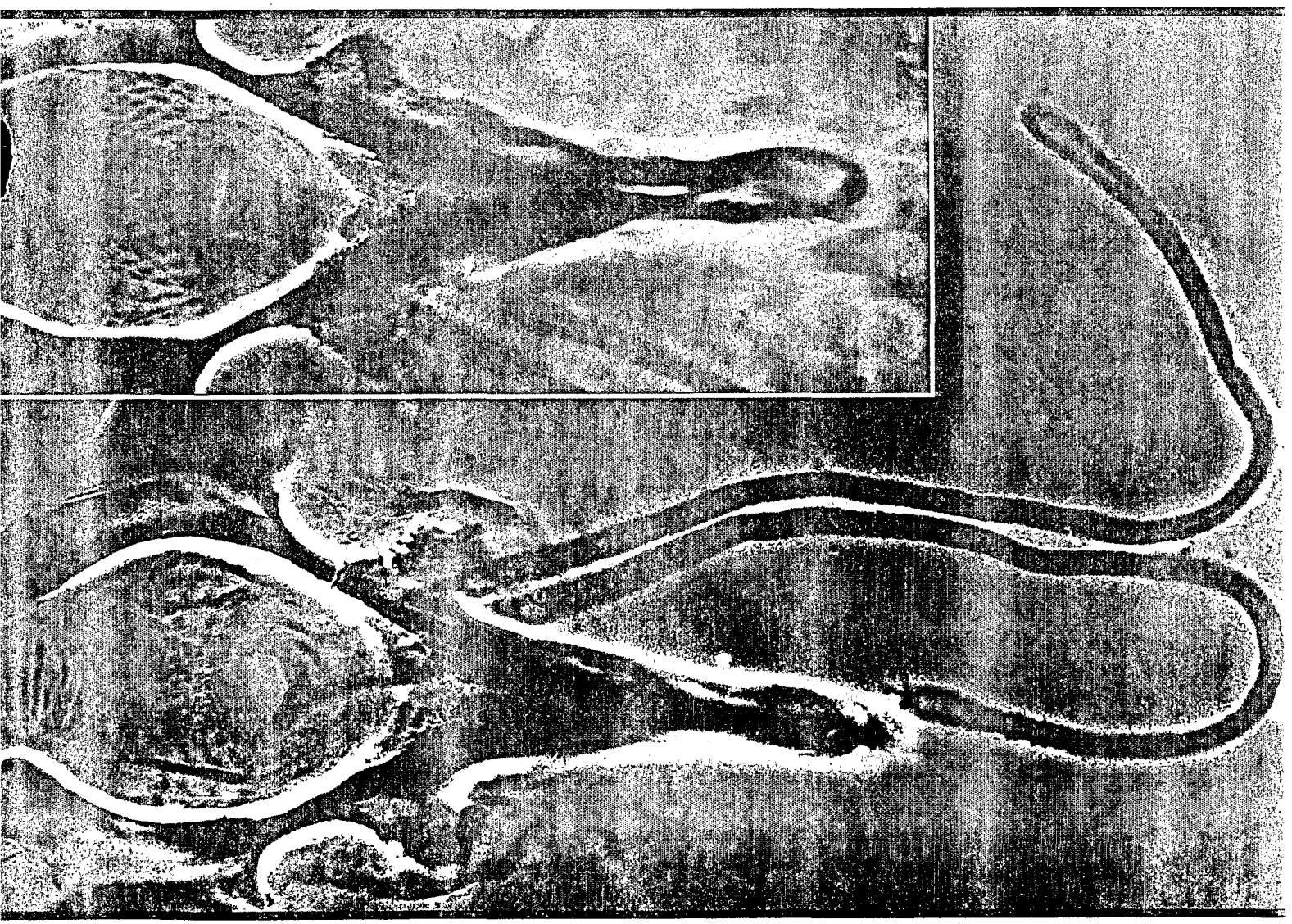
Young, D.G. & Duncan, M.A. (1994) Guide to the identification and geographic distribution of *Lutzomyia* sandflies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). *Memoirs of the American Entomological Institute*, **54**, 1059pp.

Accepted 6 July 1996

Volume 11 • Number 4 • October 1997 CODEN MVENE4 ISSN 0269-283X

Medical and Veterinary Entomology

Editors: G. B. White (Medical) and A. R. Walker (Veterinary)



Published for the Royal Entomological Society

b
Blackwell
Science