An isozymic study of *Lutzomyia longipalpis* (Diptera, Psychodidae), the vector of visceral leishmaniasis in the " Yungas " (Bolivia) [1]

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Summary

Isozyme variation was assayed in a population of Lutzomyia longipalpis, *the vector of* Leishmania chagasi *in the " Yungas " (Bolivia) in order to ascertain its taxonomic homogeneity. Although the specimens sampled exhibited some heterogeneity in their size, there was no isozyme indication of taxonomic heterogeneity within the population. The* Gpi *locus exhibited a temporal variation from February to July in the allelic frequencies and the level of heterozygosity, which may be related to climatic adaptation. Genetic variability was low, which could be explained if the present populations of* L. longipalpis *have originated from a small number of individuals (" Founder Effect ").*

Key words : Speciation — Seasonal variation — Sand flies — Epidemiology — Genetic variation — Bolivia.

Résumé

Une étude isoenzymatique chez *Lutzomyia longipalpis* (Diptera, Psychodidae), le vecteur de la leishmaniose viscérale dans les « Yungas » (Bolivie). *Nous avons étudié la variabilité isoenzymatique d'une population de* Lutzomyia longipalpis, *le vecteur de* Leishmania chagasi *dans les « Yungas » (Bolivie) dans le but de tester l'homogénéité taxonomique de cette population. Quoique les spécimens récoltés aient montré une certaine variabilité quant à leurs dimensions, les données isoenzymatiques n'ont fourni aucun indice en faveur d'une hétérogénéité taxonomique de la population considérée. le locus* Gpi *a montré une variation temporelle de février à juillet quant aux fréquences alléliques et au niveau d'hétérozygotie. Ces faits peuvent*

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être dus à une adaptation climatique. La variabilité génétique de la population considérée était faible. Une hypothèse pouvant expliquer ce résultat est que les populations actuelles de L. longipalpis *descendent d'un petit nombre d'individus (« effet fondateur »).*

Mots-clés : Spéciation — Variation saisonnière — Phlébotomes — Épidémiologie — Variation génétique — Bolivie.

Lutzomyia longipalpis, Lutz and Neiva 1912, is an anthropophilic sand fly which has been shown to be the vector of *Leishmania chagasi*, the causative agent of visceral leishmaniasis, in the " Yungas " region (department of La Paz, Bolivia) (Le Pont and Desjeux, 1985). A recent morphological study has raised the question wether *L. longipalpis* is taxonomically heterogeneous (Lane and Ward, 1984). Indeed it was observed within a population of *L. longipalpis* studied in the " Yungas " region (locality of Santa Barbara), that there is a certain degree of size heterogeneity among the specimens sampled. We report here the results of a study of isozyme variability directed towards ascertaining any possible taxonomical heterogeneity within this population.

Material and methods

The sand flies were sampled and stored in the field in liquid nitrogen in February and July 1984. Each individual was identified as *L. longipalpis* in the laboratory by morphological examination, and then immediately homogenized in 10 µl of hypotonic enzyme stabilizer (Godfrey and Kilgour, 1976). The samples so obtained were stored at — 70°C until use. Both sexes were examined in this study. Because the present work was finished before we had read the article by Lane and Ward (1984), we were unable to take into account the micromorphological characters used by these authors to suggest the existence of taxonomical heterogeneity within the species *L. longipalpis*.

Electrophoresis was carried out on cellulose acetate plates (Helena laboratories). The following enzyme systems were assayed : glucose-6-phosphate isomerase (E.C.5.3.1.9, GPI) ; α glycerophosphate dehydrogenase (E.C.1.1.1.8, α GPD) ; hexokinase (E.C.2.7.1.1, HK) ; isocitrate dehydrogenase (E.C.1.1.1.42, IDH) ; malate dehydrogenase (E.C.1.1.1.37, MDH) ; malate dehydrogenase (oxaloacetate decarboxylating) (Nadp+) or malic enzyme (E.C.1.1.1.40, ME) ; peptidase 2 (bromelain, E.C.3.4.22.4, formerly E.C.3.4.4.24, PEP 2, substrate : L-leucyl-L-alanine) ; phosphoglucomutase (E.C.5.4.2.2, formerly E.C.2.7.5.1, PGM) and 6 phosphogluconate dehydrogenase (E.C.1.1.1.44, 6PGD). The assay recipes were taken from the following authors : Kreutzer *et al.* (1983) (αGPD), Lanham *et al.* (1981) (HK) and Tibayrenc and Le Ray (1984) (all other enzymes), with a few modifications. Each enzyme system was assayed with and without its specific substrate.

Results

No color bands appeared on the plates when the specific substrate was not added to the staining solution. IDH and 6PGD gave irregular stainings and therefore are not reported in the present study.

Five of the seven enzyme systems finally considered exhibited apparently the activity of only one locus each (GPI, αGPD, HK, ME and PGM) ; one enzyme system showed the activity of two loci (MDH) ; and one showed the activity of three loci (PEP 2). Thus there are a total of ten genetic loci. Table I gives the number of specimens studied for each enzyme system.

TABLE I

Numbers of specimens studied electrophoretically in the February and July, 1984, samples of *Lutzomyia longipalpis*

Enzyme	February	July	Total
α GDH	12	90	102
GPI	71	113	184
HK	37	90	127
MDH	59	67	126
ME	59	80	139
PEP	70	70	140
PGM	62	90	152

Only two of the ten loci (*Gpi* and *Mdh1*, the locus coding for the fastest migrating MDH), appear to be polymorphic. The quaternary structure of these enzymes seems to be dimeric, given that heterozygous patterns exhibit three bands (photo 1). We found only two alleles at each of these two loci.

We have used chi-square tests to test wether the observed genotype numbers agree with the Hardy-Weinberg expectations at each of the two loci, *Gpi* and *Mdh1*. The specimens of February and July are considered separately as well as together (table II). We have pooled together the two less numerous genotypes in every instance, given that the expected numbers for at least one of them were too low (Schwartz, 1963). We had only females for the February sample, whereas both males and females were considered together for July. Other results show, however, that the genetic parameters for *Gpi* and *Mdh1* do not differ between males and females (Bonnefoy, unpublished data). The genotype numbers did not depart from the Hardy-Weinberg expectations for either *Gpi* and *Mdh1* in February, or in July, or for February and July combined.

We have calculated the allelic frequencies for *Gpi* and *Mdh1*, and the average observed and expected heterozygosity, (*H*, the probability that one individual will be heterozygous at a given locus) (table III). Using an ϵ test (comparison between two percentages ; see Schwartz, 1963, p. 58), we have compared the allelic frequencies and the observed and expected heterozygosities between the specimens caught in February and the specimens caught in July (table III). There are significant differences between February and July at the *Gpi* locus for the allelic frequencies and for the observed and expected heterozygosities. There are no significant differences at the *Mdh1* locus.

Discussion

The question has been raised whether there is taxonomical heterogeneity within the sand fly species *Lutzomyia longipalpis*, which plays an important role in the epidemiology of visceral leishmaniasis in South America. Our results suggest that the population surveyed is taxonomically homogeneous. In another taxon of sandfly (*Psychodopygus carrerai*), we have recently evidenced (Le Pont *et al.*, 1985 ; Caillard *et al.*, in press) the occurence of two cryptic species, based on the presence within the same population of alternate alleles without the corresponding heterozy-

PHOTO 1. — A cellulose acetate plate stained for the enzyme GPI, containing samples of the Phlebotomine sand fly *Lutzomyia longipalpis*. The first and last samples are from a *Trypanosoma cruzi* laboratory strain (" Tulahuen "), used for reference. Some individual sand flies exhibit 3-banded electrophorograms : this indicates that these individuals are heterozygous for the enzyme system stained, and that the enzyme has a dimeric structure. The other individuals, exhibiting 1-banded patterns, are homozygotes.

TABLE II

Chi-square tests kto test for Hardy-Weinberg equilibrium in February, July, and February and July combined for the *Gpi* and *Mdh1* genotype numbers. The numbers in parentheses are the expected numbers assuming Hardy-Weinberg equilibrium. The tests were made after combining the numbers for the two less numerous genotypes, given that the expected numbers of the rarest one are very low (Schwartz, 1963). There is 1 degree of freedom for each chi-square. All genotype numbers are consistent with HW equilibrium

Genotype	February	July	Feb. + Jul. pooled
Gpi 1/1	51 (51.5)	102 (102.2)	153 (153.4)
1/2	19 (17.9)	11 (10.5)	30 (29.2)
2/2	1 (1.6)	0 (0.3)	1 (1.4)
χ^2	0.02	0.004	0.01
Mdh 1/1	44 (45.0)	55 (54.6)	99 (99.8)
1/2	15 (13.1)	11 (11.7)	26 (24.7)
2/2	0 (1.0)	1 (0.6)	1 (1.5)
χ^2	0.09	0.01	0.03

TABLE III

Results of an ε test (Schwartz, 1963, p. 58) for comparing the allelic frequencies, and the observed and the expected heterozygosities (H) between the February and July samples for the *Gpi* and *Mdh1* loci (P is the level of significance ; significant values are underlined)

	Allelic frequencies			
Alleles	February	July	ε	P
Gpi 1	0.852	0.951	3.28	<u><0.01</u>
Gpi 2	0.148	0.049		
observed H	0.268	0.097	3.06	<u><0.01</u>
expected H	0.252	0.093	2.87	<u><0.01</u>
Mdh1 1	0.873	0.903	0.76	>0.45
Mdh1 2	0.127	0.097		
observed H	0.254	0.164	1.25	>0.22
expected H	0.222	0.175	0.66	>0.51

gotes at each of two enzyme loci, and of significant deviations from the Hardy-Weinberg expectations at three other loci. The present data are quite different : no alternate alleles without the corresponding heterozygotes are observed, and the genotype numbers for the two variable loci, *Gpi* and *Mdh1*, do not depart significantly from the Hardy-Weinberg expectations. Hence, we take it as a working hypothesis that this population belongs to the only species *L. longipalpis* and this hypothesis will be assumed in our epidemiological studies. We plan, however, to conduct further isozyme studies of other populations

of *L. longipalpis* ; and we shall take into account the micromorphological characters described by Lane and Ward (1984), in order to ascertain whether the hypothesis of taxonomical homogeneity of this species can be generalized to other Bolivian regions.

A possible explanation of the seasonal variation observed at the *Gpi* locus for the allelic frequencies and the heterozygosity (both observed and expected) is that it results from climatic adaptation ; the adaptation might not directly involve the enzyme gene loci themselves but other genes linked to them. Clearly, further studies would be required in order to decide these issues.

The observed and expected heterozygosities averaged over the ten gene loci studied are, respectively, 0.037 and 0.036 (February and July samples pooled together). These are low compared to the average heterozygosities of most insects (Ayala, 1982). A possible explanation is that the present populations of *L. longipalpis* have originated from a small number of individuals and have only recently increased greatly in numbers as a consequence of their adaptation to human environments. This hypothesis of a " Founder Effect " has been proposed to explain the low genetic variability of *Triatoma infestans*, one of the main vectors of Chagas' disease in Bolivia, and the best adapted one to human habitats (Dujardin and Tibayrenc, 1985). It is worth noting that the heterozygosity values for the sand flies *Psychodopygus carrerai* and *P. yucumensis*, which are both selvatic species, are higher than the values recorded in the present study for the peridomestic species *L. longipalpis*. For *P. carrerai*, the observed H is 0.099 and the expected H is 0.120 ; for *P. yucumensis*, these values are respectively 0.126 and 0.140 (Caillard *et al.*, in press). These values are within the typical range observed for most insects (Ayala, 1982).

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