

DIAGNOSIS BY ISOZYME METHODS OF TWO CRYPTIC SPECIES, *PSYCHODOPYGUS CARRERAI* AND *P. YUCUMENSIS* (DIPTERA: PSYCHODIDAE)¹

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Abstract. Eleven enzyme systems were used to compare 2 cryptic species previously treated as *Psychodopygus carrerai*. Two enzyme systems were each completely diagnostic. Three other loci gave evidence of reproductive isolation: the combined genotypic frequencies departed from Hardy-Weinberg equilibrium and the allele frequencies were different. The genetic distance between the 2 species is relatively small, suggesting that they are closely related.

Morphological examination of sand flies near Yucumo, Department of Beni, Bolivia, suggested the existence of 2 sympatric morphs within the taxon *Psychodopygus carrerai* (Barretto, 1946). The only external morphological difference detected is in the mesonotum coloration, which is white in the normal morph and light brown in the other morph. A preliminary isozyme study (Le Pont et al. 1985) revealed 1 enzyme system that separates the 2 sympatric morphs and provides evidence that they are 2 different species. The newly defined species was named *Psychodopygus yucumensis* Le Pont, Caillard, Tibayrenc & Desjeux, 1986. We present herein the results of a more extensive isozyme comparison of the 2 morphs that confirm that they are, indeed, different species.

MATERIAL AND METHODS

Females of both color forms were captured exclusively at ground level (*Psychodopygus* is absent

from the canopy) at only 1 site near Yucumo, always at the bottom of the same tree. Hence, all the specimens used in this study were sympatric. The captures were made during 1983 for 2 days each month from 1900-2200 h. The 2 color forms were present together throughout the year, except in October (dry season) when both forms were absent. Two collectors caught the sand flies on their arms, legs, and clothes with a mouth aspirator and put them singly into glass tubes. The sample tubes were immediately placed into liquid nitrogen in the field. The genitalia of each sand fly were dissected in the laboratory for use in identification. The head and thorax of each specimen were immediately homogenized in 10 μ l of hypotonic enzyme stabilizer (Godfrey & Kilgour 1976). The homogenates were then stored at -70 °C until used.

Electrophoresis was carried out on cellulose acetate plates (Helena Laboratories, Beaumont, Texas), with specimens of the two morphs arranged alternately on each plate. Eleven enzyme systems were studied: adenylate kinase (EC 2.7.4.3, ADK); glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PD); glucose-6-phosphate isomerase (EC 5.3.1.9, GPI); α glycerophosphate dehydrogenase (EC 1.1.1.8, α GPD); isocitrate dehydrogenase (EC 1.1.1.42, IDH); malate dehydrogenase (EC 1.1.1.37, MDH); malate dehydrogenase (oxaloacetate decarboxylating) (NADP⁺) or malic enzyme (EC 1.1.1.40, ME); peptidase 2 (bromelain, EC 3.4.22.4, formerly EC 3.4.4.24, substrate: leucyl-L-alanine); phosphoglucomutase (EC 5.4.2.2, formerly EC 2.7.5.1, PGM); 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6PGD); and xanthine dehydrogenase (EC 1.2.1.37, XDH). The tank buffers and staining recipes were as previously described (Le Pont et al. 1985). Each enzyme system was assayed with and without its specific substrate.

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Genetic distance calculations and statistical analysis

TABLE 1. Characteristics of the 11 loci surveyed and of their enzyme products when the data of *Psychodopygus carrerai* and *P. yucumensis* are jointly considered.

Enzyme locus	State	No. alleles	Quaternary structure	No. species
<i>Adk 1</i>	polymorphic	2	monomeric	167
<i>Gpi</i>	polymorphic	3	dimeric	220
<i>Idh</i>	polymorphic	2	monomeric	178
<i>Mdh</i>	polymorphic	3	dimeric	253
<i>Pep 2*</i>	polymorphic	2	?	102
<i>Pgm</i>	polymorphic	5	monomeric	245
<i>Xdh*</i>	polymorphic	2	?	163
<i>Adk 2</i>	monomorphic	1	?	167
<i>αGpd</i>	monomorphic	1	?	132
<i>G6pd</i>	monomorphic	1	?	120
<i>6Pgd</i>	monomorphic	1	?	149

* *Pep 2* and *Xdh* are polymorphic when both species are combined, but monomorphic for each species separately.

RESULTS

Table 1 summarizes the results obtained when the samples of the 2 cryptic species were compared. No color bands appeared on the plates when the

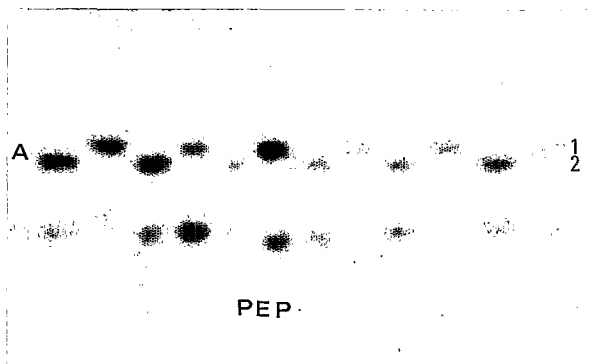


FIG. 1. An electrophoretic plate stained for peptidase. There are 2 zones of activity, but only the fastest one (labeled "A") is considered. Specimens of the 2 species have been placed alternately on the plate: the 1st individual on the left is *P. yucumensis*, the next one is *P. carrerai*, and so on. This enzyme system is fully diagnostic between the 2 species. No heterozygous patterns were found.

Significant differences in allelic frequencies were observed at the 3 highly polymorphic loci *Gpi*, *Mdh*, and *Pgm*; at the other 2 loci, the frequencies of the

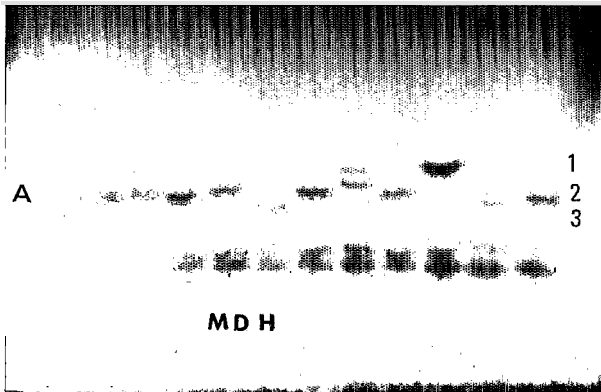


FIG. 3. A plate stained for malate dehydrogenase. Only the fastest migrating zone of activity (A) is considered. Specimens of the 2 species are alternately arranged along the plate. Three alleles were found. On this plate, it would seem that allele 2 is different for the 2 species, but we were unable to ascertain this with other assays. The heterozygotes exhibit a 3-banded phenotype, indicating that this enzyme is a dimer.

phic loci (*Mdh*: $\chi^2 = 40.8$, 5 df, $P < 0.001$; *Pgm*: $\chi^2 = 47.8$, 12 df, $P < 0.001$), but not at the other 3.

We calculated the average heterozygosity, H (the probability that 1 individual will be heterozygous at a given locus), for the 11 gene loci studied. For *P. carrerai*, the observed $H = 0.099$ and the expected $H = 0.120$; for *P. yucumensis*, observed $H = 0.126$, expected $H = 0.145$. These heterozygosities were within the typical range observed for insects (Ayala 1982), although the number of loci sampled was small.

DISCUSSION

The present results further distinguish the 2 color morphs as 2 separate species, *P. carrerai* and *P. yucumensis*. Two loci, *Pep 2* and *Xdh*, are fully diagnostic between the 2 species; no common alleles were found in more than 80 individuals of each species examined at each locus. We calculated the probability of incorrect diagnosis of the species using the 3 highly polymorphic loci (Ayala & Powell 1972). The probabilities of making the wrong identification were 0.398 for *Gpi*, 0.233 for *Mdh*, and 0.324 for *Pgm*. When these 3 polymorphic loci are used jointly, the probability of correct diagnosis of the species for a single individual is 0.97. Thus, the 3 loci may be used jointly for diagnosis with only a 0.03 probability of error, although the existence of the 2 fully diagnostic loci would normally make superfluous the use of the polymorphic loci.

The differences in allele frequencies are re-

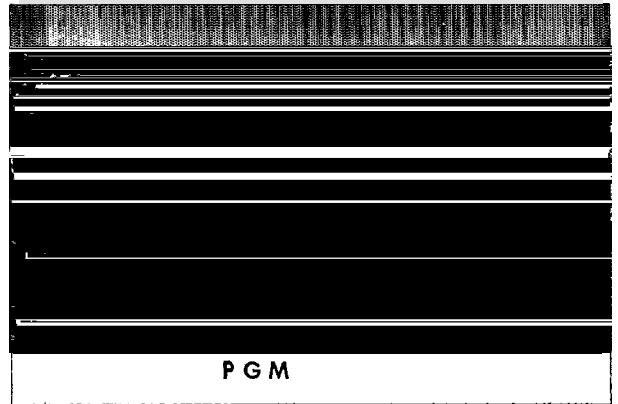


FIG. 4. A plate stained for phosphoglucumutase. Specimens of the 2 species are placed alternately along the plate. Five alleles are apparent. The 2-banded pattern of the heterozygotes indicates that this enzyme is a monomer.

vealed also by use of standard deviate statistics (Table 2) and by the significant departure from Hardy-Weinberg expectations for the pooled genotypic data at 2 of the 3 loci. At the *Pgm* locus, the *P. carrerai* population departs from the Hardy-Weinberg frequencies. This might be due to natural selection or to lack of random mating, which in turn could be the result of recent gene flow or of the presence of 2 or more reproductively isolated populations within *P. carrerai*.

The existence of 2 sympatric species of *Psychodopygus* in this region of Bolivia is of epidemiolog-

TABLE 2. Allele frequencies at 5 polymorphic loci in *Psychodopygus carrerai* and *P. yucumensis*. The similarity of the allele frequencies is examined by the standard deviate statistic ϵ .

Locus and allele	<i>carrerai</i>	<i>yucu-mensis</i>	ϵ	P
<i>Adk 1 1</i>	1	0.98	—*	—
<i>Adk 1 2</i>	0	0.02	—	—
<i>Gpi 1</i>	0.123	0.014	4.54	<0.001
<i>Gpi 2</i>	0.854	0.904	1.61	NS
<i>Gpi 3</i>	0.023	0.082	2.79	<0.02
<i>Idh 1</i>	0.017	0.028	—	—
<i>Idh 2</i>	0.983	0.972	—	—
<i>Mdh 1</i>	0.056	0.295	7.06	<0.001
<i>Mdh 2</i>	0.869	0.472	9.47	<0.001
<i>Mdh 3</i>	0.075	0.232	4.89	<0.001
<i>Pgm 1</i>	0.189	0.012	6.59	<0.001
<i>Pgm 2</i>	0.223	0.159	1.80	NS
<i>Pgm 3</i>	0.235	0.365	3.13	<0.02
<i>Pgm 4</i>	0.273	0.377	2.45	<0.02
<i>Pgm 5</i>	0.080	0.087	0.28	NS

* ϵ was not calculated for *Adk 1* and *Idh* because the allele frequencies in both species are very close to 1 or 0. NS = not significant ($P > 0.05$).

TABLE 3. Genotypic numbers at 5 polymorphic loci in 2 sympatric species of sand flies, *Psychodopygus carrerai* and *P. yucumensis*. The fastest migrating allele is designated "1," the next fastest "2," and so on.

Genotype	carre- rai	yucu- mensis	Genotype	carre- rai	yucu- mensis
<i>Adk</i> 1 1/1	83	81	<i>Pgm</i> 1/1	9	1
<i>Adk</i> 1 1/2	0	3	<i>Pgm</i> 1/2	7	1
<i>Adk</i> 1 2/2	0	0	<i>Pgm</i> 1/3	15	0
<i>Idh</i> 1/1	1	0	<i>Pgm</i> 1/4	3	0
<i>Idh</i> 1/2	1	5	<i>Pgm</i> 1/5	2	0
<i>Idh</i> 2/2	87	84	<i>Pgm</i> 2/2	7	5
<i>Gpi</i> 1/1	3	1	<i>Pgm</i> 2/3	17	20
<i>Gpi</i> 1/2	21	1	<i>Pgm</i> 2/4	15	6
<i>Gpi</i> 1/3	0	0	<i>Pgm</i> 2/5	0	3
<i>Gpi</i> 2/2	81	90	<i>Pgm</i> 3/3	7	18
<i>Gpi</i> 2/3	5	18	<i>Pgm</i> 3/4	8	32
<i>Gpi</i> 3/3	0	0	<i>Pgm</i> 3/5	2	4
<i>Mdh</i> 1/1	1	16	<i>Pgm</i> 4/4	16	24
<i>Mdh</i> 1/2	7	24	<i>Pgm</i> 4/5	7	9
<i>Mdh</i> 1/3	5	19	<i>Pgm</i> 5/5	4	3
<i>Mdh</i> 2/2	99	36			
<i>Mdh</i> 2/3	14	24			
<i>Mdh</i> 3/3	0	8			

ical interest, given that the species may be *Leishmania* vectors. The *Leishmania* stocks isolated from the more recently described sand fly species have electrophoretic patterns typical of *Leishmania braziliensis braziliensis* (Desjeux & Le Pont, unpubl. data) and, hence, indicate that *P. yucumensis* is probably a human *Leishmania* vector. The case is different for *P. carrerai*, which yielded an electrophoretically untypical *Leishmania* stock. We have work in progress to ascertain whether these species are able to transmit *Leishmania*.

The genetic distance (D) between the 2 cryptic species is, for all 11 gene loci, 0.256. [D estimates the average number of codon substitutions per gene that have occurred since the phylogenetic divergence of the 2 species (Nei 1972).] This degree of genetic divergence between 2 species is relatively low for insects, even when the species are cryptic (Ayala 1982). This suggests that the evolutionary divergence of the 2 species has occurred in a relatively recent past, which is of course consistent with their great morphological similarity. Nevertheless, we should reiterate that 2 of the 11 loci surveyed are fully diagnostic for these 2 species.

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