Preliminary Evaluation of the Genetic Relatedness of Three Species of the Subgenus *Dendromyia* Theobald and Other Species of the Genus *Wyeomyia* Theobald (Diptera: Culicidae)

Monique Albuquerque Motta/+, Ricardo Lourenço-de-Oliveira, Fernando Araujo Monteiro*, Luciana Ramos Barros

Departamento de Entomologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil
*Departamento de Genética, Universidade Federal do Rio de Janeiro, Av. Brigadeiro Trompowsky, 21941-590 Rio de Janeiro, RJ, Brasil

An eletrophoretic analysis of three species of the subgenus Dendromyia (Wyeomyia luteoventralis, Wy. ypsipola *and* Wy. testei) *and three species belonging to different groups in the genus* Wyeomyia (Wy. negrensis, Wy. mystes *and* Wy.confusa) *was performed. Eight enzyme loci were analyzed. High values of genetic identity were detected among the species of the subgenus* Dendromyia: Wy. luteoventralis, Wy. ypsipola *and* Wy. testei *(mean value 0.63). On the other hand low values of genetic identity were observed among* Wy. negrensis, Wy. mystes *and* Wy. confusa *(mean value 0.23), suggesting that they belong, at least, to distinct subgenera within the Genus* Wyeomyia. *The UPGMA phenogram revealed the grouping of the* Dendromyia *species, while the others clustered at lower identity levels.*

Key words: mosquitoes - *Wyeomyia* - *Dendromyia* - allozyme - genetic identity

Most Neotropical Sabethini belong to the genus *Wyeomyia* Theobald. These diurnal sylvatic mosquitoes may be vectors of arboviruses in the Neotropical region (Hervé et al. 1986). The subgenus *Dendromyia* Theobald holds most species in the genus *Wyeomyia*. The subgenus *Dendromyia* was previously considered to be composed of 43 species, the majority of which were superficially described from only a few specimens. In general, the immature stages and the morphological characters of males and females of *Dendromyia* species are poorly known or are unknown. Therefore the phylogenetic relationships among species in the subgenus *Dendromyia* remain obscure.

Currently, six nominal species are recognized as belonging to the subgenus *Dendromyia*: *Wy. luteoventralis* Theobald (the type-species of *Dendromyia*), *Wy. ypsipola* Dyar, *Wy. jocosa* Dyar & Knab, *Wy. testei* Senevet & Abonnenc, *Wy. trifurcata* Clastrier and *Wy. complosa* Dyar (Motta & Lourenço-de-Oliveira 1995). An extensive morphological analysis and redescription of immature stages as well as both males and females of these six species (unpublished data) showed that they share more characters than the group of six species shares with other *Wyeomyia* species. The purpose of this study is to perform a comparative allozyme analysis and to estimate genetic similarities among these species and other related *Wyeomyia* species. The level of genetic similarity is expressed by identity values (**I**) (Nei 1972), and can be used to determine taxonomic relationships. The technique of allozyme eletrophoresis has been successfully and widely used to assist in resolving taxonomic problems and to infer on genetic relationships (Bullini 1982, Thorpe & Solé-Cava 1994). In the present report we compare six species, three from the subgenus *Dendromyia* and three other species previously included in different groups or series in the subgenus *Dendromyia*, as proposed by Lane and Cerqueira (1942).

MATERIALS AND METHODS

Multilocus enzyme eletrophoresis was carried out in agarose gels with adult specimens, as described by Momen and Salles (1985) with the modifications of Rosa-Freitas (1988).

The allozyme study compared three species of *Dendromyia* (*Wy. luteoventralis, Wy. ypsipola* and *Wy. testei*) and three species belonging to the following groups or "series" (Lane & Cerqueira 1942): series Prosopolepis (*Wy. confusa* Lutz), series Cleobonnea (*Wy. negrensis* Gordon & Evans) and series Dendromyia (*Wy. mystes* Dyar).

The allozyme analysis included field collected larvae and adults (on human bait), in the follow-

+Corresponding author. Fax: + 55-21-290.9339
Received 11 June 1997
Accepted 23 January 1998

ing localities in Brazil: Porto Velho, State of Rondônia; Manaus, State of Amazonas; Belém, State of Pará; Peixoto de Azevedo, State of Mato Grosso; Itaguaí, Nova Friburgo and Guapimirim, State of Rio de Janeiro; São Paulo, State of São Paulo, and Joinville, State of Santa Catarina. The number of individuals analyzed varied according to species and locality (Table I).

Twenty-six enzymes were tested in different buffer systems: citric buffer (ODH, ALDH, ADH, ACP, LEDH, LDH, ACON, GDH, MPI, G6PDH, PEP, PEPD, a EST, NH and HK); maleic buffer (ADH, MPI, G6PDH, PEP1, HK and HBDH); and phosphate buffer (LEDH, MPI, G6PDH, PEP2, NH, GOT and HBDH). Only seven of the enzymes were scorable, by producing sharp, defined bands comprising a total of eight enzymatic *loci*. Enzymes and buffer systems used were: (1) tris - maleic buffer 0.1 M (pH 7.4): malic enzyme 1.1.1.40 (ME), isocitrate dehydrogenase 1.1.1.42 (IDH) and phosphoglucomutase 2.7.5.1. (PGM); (2) phosphate buffer 0.2 M (pH 8.0): malate dehydrogenase 1.1.1.37 (MDH), glucose phosphate isomerase 5.3.1.9 (GPI) and fumarate hydratase 4.2.1.2 (FUM) and (3) tris citric 0.2 M (pH 8.1): 6-phosphogluconate dehydrogenase 1.1.1.44 (6 PGDH).

Allozyme data were analysed using the genetic computer program BIOSYS - 1 (Swofford & Selander 1981). Levels of heterozygosity and genetic distances and identities (Nei 1978) were estimated for all species analyzed. The genetic identities were then used to build a phenogram using the unweighted pair group mean analysis (UPGMA) (Sneath & Sokal 1973).

RESULTS

One out of the eight *loci* analyzed - GPI - was monomorphic in all species. FUM was monomorphic in five species except in *Wy. confusa*, while PGM was polymorphic in all species (Table I).

Enzymes that presented polymorphic *loci* were: in *Wy. testei* (PGM, IDH 1 and 2, ME and 6-PGDH), *Wy. negrensis* (MDH, PGM, IDH 1 and 2 and ME) and *Wy. mystes* (MDH, PGM, IDH 2, ME and 6 PGDH). The frequency of polymorphic *loci* was 62% for these three species; the mean heterozygosity values were 0.13, 0.08 and 0.13, and mean number of alleles per *locus* were 2.3, 1.9 and 2.3, respectively (Table II).

Except for GPI, the majority of enzymes was polymorphic (87.5%) in *Wy. confusa*, with mean heterozygosity of 0.082 and a mean number of 2.6 alleles per *locus*. In *Wy. luteoventralis* six *loci* were polymorphic (MDH, PGM, IDH 1 and 2, ME and 6 PGDH) and the percentage of polymorphic *loci* was 75%, with the mean number of 2.0 alleles per *locus* and the observed mean heterozigozity of 0.23, which was the highest among the analyzed species. *Wy. ypsipola* presented only one polymorphic *locus* (PGM), and the values were 12.5, 0.042 and 1.3 respectively (Table II).

Values of genetic identities (I) and distances (D) (Nei 1978) between the species are listed in Table III. High values of genetic identity were detected among the *Dendromyia* species - *Wy. testei*, *Wy. luteoventralis* and *Wy. ypsipola* (mean value 0.63). On the other hand, low **I** values were observed among *Wy. negrensis*, *Wy. mystes* and *Wy. confusa* (mean value 0.23). The UPGMA phenogram constructed with identity values demonstrated a grouping of *Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*, while other species clustered at lower identity levels.

DISCUSSION

This is the first allozyme analysis performed with Neotropical species of *Wyeomyia*. In the course of this study, we found that several enzyme systems used in similar analyses of other culicid mosquitoes (*Anopheles*, Rosa-Freitas et al. 1990, Narang et al. 1991; *Culex*, Humeres et al. 1990; *Aedes*, Nielsen et al. 1995) did not produce readable bands.

The limited number of enzymatic *loci* analyzed may not provide the best estimate of variation that actually exists between the assayed species. Regardless, the results of the allozyme analysis still agreed with the morphological studies and the subsequent taxonomic treatment by Motta and Lourenço-de-Oliveira (1995) for species belonging to the subgenus *Dendromyia*.

The highest percentage of polymorphic *loci* (Table II) was observed in *Wy. confusa* (87.5%). The percentage of polymorphic *loci* in this species is higher than those found in some mosquitoes, such as *An. albimanus* (55%, according to Narang et al. 1991), *An. aquasalis* (27%, Flores-Mendoza 1994) and *An. pseudopunctipennis* (ranging from 12.1% to 78.8%, Manguin et al. 1995). Alternatively, *Wy. ypsipola* presented a low percentage of polymorphic *loci* (12.5%).

The mean heterozygosity values found for the analyzed *Wyeomyia* species are within the range observed for other mosquito groups, such as *Anopheles* (Narang et al. 1991, Flores-Mendoza 1994).

Among the species currently included in the subgenus *Dendromyia* (*Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*), high values of genetic identity were obtained (Table III). This reinforces the conclusion from morphological analysis, that these three species comprise a distinct group within the genus *Wyeomyia*. In addition, the highest **I** value

TABLE I

Allele frequencies for polymorphic enzyme *loci* in *Wyeomyia negrensis*, *Wy. mystes*, *Wy. confusa*, *Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*. Number of specimens tested in parenthesis

	Species and populations					
Locus	*Wy. negrensis* Manaus (14) Peixoto de Azevedo (27) Belém (6)	*Wy. mystes* Itaguaí (39)	*Wy. confusa* São Paulo (2) Nova Friburgo (7) Guapimirim (14) Joinville (17)	*Wy. luteoventralis* Belém (6)	*Wy. ypsipola* Belém (2) Porto Velho (4) Peixoto de Azevedo (3)	*Wy. testei* Porto Velho (38)
MDH	(39)	(39)	(37)	(3)	(9)	(37)
A	.013	.000	.000	.000	.000	.000
B	.987	.064	.014	.500	.000	.000
C	.000	.000	.986	.000	.000	.000
D	.000	.936	.000	.500	1.000	1.000
PGM	(32)	(30)	(25)	(5)	(9)	(32)
A	.000	.000	.020	.000	.000	.188
B	.000	.000	.000	.000	.000	.203
C	.891	.000	.040	.000	.000	.000
D	.000	.000	.140	.100	.111	.531
E	.078	.000	.780	.600	.389	.016
F	.031	.900	.000	.000	.500	.016
G	.000	.050	.020	.300	.000	.047
H	.000	.050	.000	.000	.000	.000
IDH1	(41)	(26)	(39)	(5)	(9)	(38)
A	.000	1.000	.000	.000	.000	.013
B	.122	.000	.013	.300	.000	.000
C	.000	.000	. 987	.700	1.000	.974
D	.854	.000	.000	.000	.000	.013
E	.024	.000	.000	.000	.000	.000
IDH2	(40)	(39)	(39)	(5)	(9)	(35)
A	.3l3	.000	.026	.400	1.000	.000
B	.688	.526	.962	.400	.000	.000
C	.000	.462	.013	.000	.000	.029
D	.000	.000	.000	.200	.000	.971
E	.000	.013	.000	.000	.000	.000
ME	(37)	(39)	(30)	(6)	(9)	(34)
A	.081	.000	.000	.000	.000	.000
B	.000	.000	.033	.000	.000	.000
C	.919	.013	.000	.833	.000	.206
D	.000	.013	.000	.167	1.000	.794
E	.000	.026	.967	.000	.000	.000
F	.000	.949	.000	.000	.000	.000
FUM	(31)	(32)	(30)	(2)	(9)	(30)
A	.000	1.000	.000	.000	.000	.000
B	.000	.000	.100	1.000	1.000	1.000
C	.000	.000	.900	.000	.000	.000
D	1.000	.000	.000	.000	.000	.000
GPI	(31)	(31)	(30)	(6)	(9)	(28)
A	.000	.000	.000	.000	.000	1.000
B	1.000	1.000	1.000	1.000	1.000	.000
6PGD	(31)	(30)	(26)	(4)	(9)	(25)
A	.000	.000	.058	.000	.000	.000
B	.000	.000	.904	.500	.000	.940
C	1.000	.017	.000	.000	.000	.000
D	.000	.917	.019	.500	1.000	.060
E	.000	.067	.019	.000	.000	.000

TABLE II

Measures of genetic variation of *Wyeomyia negrensis*, *Wy. mystes*, *Wy. confusa*, *Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*

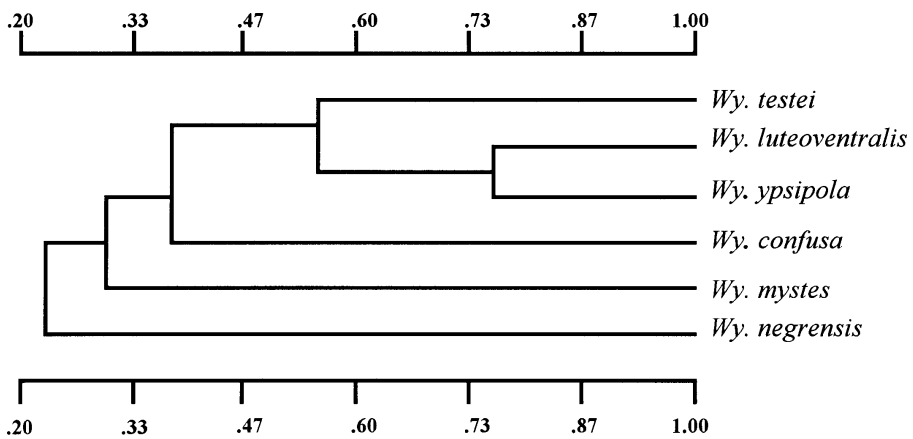
Species	Mean sample size per *locus*	Mean No. of alleles per *locus*	Percentage of *loci* polymorphic [a]	Mean heterozygosity	
				Direct-count	HdyWbg expected [b]
Wy. negrensis	35.3	1.9	62.5	.081	.134
	(1.6)	(.3)		(.045)	(.056)
Wy. mystes	33.3	2.3	62.5	.127	.136
	(1.8)	(.4)		(.057)	(.061)
Wy. confusa	32.0	2.6	87.5	.082	.117
	(2.0)	(.5)		(.027)	(.044)
Wy. luteoventralis	4.5	2.0	75.0	.233	.407
	(.5)	(.3)		(.079)	(.098)
Wy. ypsipola	9.0	1.3	12.5	.042	.078
	(.0)	(.3)		(.042)	(.078)
Wy. testei	32.4	2.3	62.5	.133	.151
	(1.6)	(.6)		(.074)	(.081)

[a]: a *locus* is considered polymorphic if more than one allele was detected; [b]: unbiased estimate (see Nei 1978).

TABLE III

Estimates of Nei's (1978) unbiased genetic identity (above diagonal) and genetic distance (below diagonal) for *Wyeomyia negrensis*, *Wy. mystes*, *Wy. confusa*, *Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*

Species	1	2	3	4	5	6
1 *Wy. negrensis*	*****	.214	.255	.478	.190	.029
2 *Wy. mystes*	1.542	*****	.223	.383	.464	.153
3 *Wy. confusa*	1.368	1.502	*****	.543	.339	.289
4 *Wy. luteoventralis*	.738	.959	.610	*****	.762	.573
5 *Wy. ypsipola*	1.661	.768	1.080	.272	*****	.551
6 *Wy. testei*	3.525	1.880	1.241	.556	.596	*****



Phenogram of genetic relationships among *Wyeomyia negrensis*, *Wy. mystes*, *Wy. confusa*, *Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*, using unweighted pair-group (with arithmetic mean) clustering of Nei (1978) identities.

was obtained between *Wy. luteoventralis* and *Wy. ypsipola* (**I** = 0.76). In spite of *Wy. luteoventralis* and *Wy. ypsipola* having very distinct external male and female characters, the morphological characters of the immature stages are nearly identical.

On the other hand, the allozyme analyses of *Wy. negrensis*, *Wy. mystes*, *Wy. confusa* (belonging to taxonomically undetermined subgenera) and the above mentioned members of the subgenus *Dendromyia* (*Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*) showed low genetic identity among them, with **I** values ranging from 0.03 to 0.54. This also agrees with the current taxonomic treatment of the genus *Wyeomyia* based on morphological analysis by Motta and Lourenço-de-Oliveira (1995) and Motta (1996). The highest **I** value found between a member of the subgenus *Dendromyia* and a non *Dendromyia* species was that between *Wy. luteoventralis* and *Wy. confusa* (0.54). Indeed, *Wy. confusa* is bionomically similar to the *Dendromyia* species, e.g. predatory behavior of larvae and use of *Callathea* and *Heliconia* plants as larval habitats. Regardless, *Wy. confusa* belongs to the Prosopolepis group of *Wyeomyia* because it is neither morphologically related nor has enough genetic similarity with other members of the subgenus *Dendromyia*.

In reviews on the relationship of **I** to systematic diversity of vertebrates, invertebrates and plants, Thorpe (1982) and Thorpe and Solé-Cava (1994) concluded that generally conspecific populations have **I** values above 0.85, while between congeneric species the usual range is about 0.85 to 0.30 and between genera **I** values are usually in the range from 0.45 to zero (generally bellow 0.35). The **I** values observed among *Wy. luteoventralis*, *Wy. ypsipola* and *Wy. testei*, ranging from 0.55 to 0.76, show that they are closely related species and reinforce their congeneric relationship. On the other hand, the allozyme analysis of *Wy. negrensis*, *Wy. mystes* and *Wy. confusa* presented low genetic identity with **I** values ranging from 0.21 to 0.26, suggesting that they belong to distinct groups, probably different subgenera within the genus *Wyeomyia*. Nevertheless, the **I** values between some of them were very low, such as between *Wy. testei* and *Wy. negrensis* (**I** = 0.03). These results suggest that they may belong to different genera. Indeed, *Wy. negrensis*, *Wy. confusa* and *Wy. mystes* were previously thought to belong to the series Cleobonnea, Prosopolepis and Dendromyia respectively (Table III). Our findings suggest that these three series consisted of an unnatural classification, and that more detailed and comprehensive morphological and genetic studies should be conducted to better understand the phylogenetic relationships among the *Wyeomyia* species.

ACKNOWLEDGEMENTS

To Dr MG Rosa-Freitas and M Monteiro for suggestions in the manuscript; Drs H Momen, R Pacheco and NA Honório for the sugestions and support in the laboratory; Dr AT Rosa and OV Silva, Evandro Chagas Institute, for the support on the field.

REFERENCES

Bullini L 1982. Enzyme variants in the identification of parasites and vectors: methodological aspects of the electrophoretic approach, p. 53-69. In BN Newton, F Michal (eds), *New Aproaches to the Identification of Parasites and Their Vectors*, WHO, Basel, Switzerland.

Flores-Mendoza CF 1994. *Um Estudo Sobre Alguns Aspectos Comportamentais, Morfológicos e Bioquímicos de Anopheles aquasalis Curry, 1932*, MSc Thesis, Instituto Oswaldo Cruz, Rio de Janeiro, 172 pp.

Hervé JP, Dégallier N, Travassos da Rosa APA, Pinheiro FP, Sá Filho GC 1986. Arboviroses - Aspectos ecológicos, p. 409-437. In *Instituto Evandro Chagas - 50 Anos de Contribuição às Ciências Biológicas e à Medicina Tropical*, Vol I., Fund Serv Saúde Pública, Belém.

Humeres SG, Gardenal CN, Almirón W, Sereno R, Sabattini MS 1990. *Culex* species (Diptera: Culicidae) from Central Argentina identification by electrophoretic zymograms and genetic relationships. *J Med Entomol 27*: 784-788.

Lane J, Cerqueira NL 1942. Os sabetíneos da América (Diptera, Culicidae). *Arch Zool S Paulo 3*: 473-849.

Manguin S, Roberts DR, Peyton EL, Fernandez-Salas I, Barreto M, Loayza RF, Spinola RE, Granaou RM, Rodriguez MH 1995. Biochemical systematics and population genetic structure of *Anopheles pseudopunctipennis*, vector of malaria in Central and South America. *Am J Trop Med Hyg 53*: 362-377.

Momen H, Salles CA 1985. Enzyme markers for *Vibrio cholerae*: identification of classical, eltor and environmental strains. *Trans R Soc Trop Med Hyg 79*: 773-776.

Motta MA 1996. *O Subgênero Dendromyia Theobald (DIPTERA: CULICIDAE): Um Estudo Morfológico e Bioquímico*, MSc Thesis, Instituto Oswaldo Cruz, Rio de Janeiro, 121 pp.

Motta MA, Lourenço-de-Oliveira R 1995. *Wyeomyia luteoventralis* Theobald, the type species of the subgenus *Dendromyia* Theobald (Diptera: Culicidae). *Mem Inst Oswaldo Cruz 90*: 375-385.

Narang SK, Seawright JA, Suarez MF 1991. Genetic structure of natural populations of *Anopheles albimanus* in Colombia. *J Am Mosq Control Assoc 7*: 437-445.

Nei M 1972. Genetic distance between populations. *Amer Natur 106*: 283-292.

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

Nielsen BO, Loeschcke V, Simonsen V 1995. Separating female *Aedes cantans* and *Aedes annulipes* by

morphology and allozymes (Diptera: Culicidae). *Mosq Syst* 27: 100-109.

Rosa-Freitas MG 1988. *Anopheles albitarsis Lynch-Arribálzaga, 1878 (Diptera; Culicidae): Um Estudo Comparativo de Caracteres Morfológicos, Bioquímicos e Comportamentais de Populações de Dez Localidades*, MSc Thesis, Instituto Oswaldo Cruz, Rio de Janeiro, 105 pp.

Rosa-Freitas MG, Deane LM, Momen H 1990. A morphological, isoenzymatic and behavioural study of ten populations of *Anopheles (Nyssorhynchus) albitarsis* Lynch-Arribalzaga, 1878 including from the type-locality, Baradero, Argentina. *Mem Inst Oswaldo Cruz 85*: 275-289.

Swofford DL, Selander RB 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of eletrophoretic data in population genetics and systematics. *J Hered 72*: 281-283.

Sneath PHA, Sokal RR 1973. *Numerical Taxonomy*, Freeman, San Francisco, 573 pp.

Thorpe JP 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Annu Rev Ecol Syst 13*: 139-168.

Thorpe JP, Solé-Cava AM 1994. The use of allozyme electrophoresis in invertebrate systematics. *Zool Scr 23*: 3-18.