

## Isoenzymatic polymorphisms in urban populations of *Drosophila willistoni*

### Polimorfismo isoenzimático en poblaciones urbanas de *Drosophila willistoni*

CARMEN C. R. SAAVEDRA, MARLY NAPP, MARIA LUIZA REGULY & VERA LUCIA S. VALENTE<sup>1</sup>

<sup>1</sup>Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, CEP 91501-970, Porto Alegre, RS, Brazil, e-mail: valente@if1.if.ufrgs.br

#### ABSTRACT

Natural populations of *Drosophila willistoni* collected both in urban sites and in the wild, as control, were analysed with respect to their isoenzyme polymorphisms in six loci of enzyme systems, through horizontal electrophoresis. The first pool of populations was collected and analysed during the years 1987 and 1988, and the other during 1992 and 1993, in comparable seasons. The level of enzymatic polymorphism first detected was smaller in comparison to that observed four years later, suggesting an increase of variability along the time elapsed since the first sampling of this species, in the city of Porto Alegre. Our findings are suggestive of an increasing adjustment of the species to this new environment.

**Key words:** *Drosophila willistoni*, urban populations, isoenzymes.

#### RESUMEN

Poblaciones naturales de *Drosophila willistoni* recolectadas en lugares urbanos y silvestres, como control, fueron analizadas según su polimorfismo isoenzimático en seis loci de sistemas enzimáticos, mediante electroforesis horizontal. El primer grupo de poblaciones fue obtenido y estudiado en los años 1987 y 1988, mientras el segundo lo fue en los años 1992 y 1993, en períodos del año comparables. El nivel de polimorfismo enzimático detectado en el primer período fue pequeño en comparación con el detectado en el segundo grupo de muestras, obtenidas cuatro años después, sugiriendo un incremento de variabilidad temporal. Nuestros hallazgos sugieren un ajuste creciente de la especie a su nuevo ambiente.

**Palabras clave:** *Drosophila willistoni*, poblaciones urbanas, isoenzimas.

#### INTRODUCTION

Marginal populations present a unique opportunity for evolutionary studies, since it is under such conditions that genetic adaptation to extreme environments could more frequently be found. In the Genus *Drosophila*, the genetics of marginal populations has been the matter of several classical studies (Da Cunha & Dobzhansky 1954, Da Cunha et al. 1959, Carson 1955, 1959, Dobzhansky 1965).

*Drosophila willistoni* is a wild, widespread species, native to hot and humid Neotropical forests, occurring from Florida and Mexico, that corresponds to the Northern range of its distribution, to Argentina, in the South (Spassky et al. 1971, Cordeiro & Winge 1995). Although classified as a "wild" species (Dobzhansky 1965, Carson 1965), *D. willistoni* seems to be capable of exploring man-altered environments (Dobzhansky 1965), due to its wide genetic variability expressed through several genetic

markers until now surveyed. These include enzyme variability, documented by Borba & Napp (1986) for samples from southern latitudes. This potentiality was confirmed by the finding of this fly in samples from urban places in the city of Porto Alegre of southern Brazil, by Valente & Araújo (1986) and by Goñi et al. (1997, 1998) in urban and suburban places of the city of Montevideo, in Uruguay. The present report is an attempt to describe the level of genetic variability of these urban populations of *D. willistoni*.

#### MATERIAL AND METHODS

Samples of natural populations of *Drosophila willistoni* were collected flying around banana baits, or as they emerged from rotten fruits of native and exotic plants, in parks and squares of Porto Alegre city (30° 10' S, 51° 06' W), as well as in a wild control site, Eldorado do Sul (30° 05' S, 51° 30' W) 40 km distant.

Fermented fruits colonised by preadult forms of *Drosophila* were carried to the laboratory and individually placed in tubes with culture medium (Marques et al. 1966) until the emergence of adult flies, according to Brncic & Valente (1978). The emerged flies were aspirated and classified by inspection of their distinctive characteristics of external genitalia. Isofemale lines of *D. willistoni* were immediately established, with one F1 larva per female dissected, processed according to Ashburner (1967) and analysed with respect to its chromosomal polymorphism (Valente et al. 1993). Following this routine, through the analysis of the polytene chromosomes, we also discarded the possibility of misidentifications, since the sibling species *D. paulistorum* is sympatric to *D. willistoni* in almost all our samples. After their emergence, the flies were aged for a week, homogenized, and subjected to horizontal electrophoresis assays.

Adult individuals of both sexes were submitted to horizontal electrophoresis in polyacrilamide gels revealed for the following enzymatic systems, under their respective conditions. Octanol

dehydrogenase (Odh) and Malate dehydrogenase (Mdh): buffers according to Poulik (1957); 8 % and 6 % concentration of the gels, respectively, and staining according to Ayala et al. (1972). Acid phosphatase (Acph) and  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -Gpdh): Hüetzel & Bush (1972) buffers, gels at 6 % and staining by Ayala et al. (1972). Amylase (Amy): buffer of Scandalios (1969), 8 % concentrated gels.

Expected heterozygosities per locus (Ayala et al. 1972) were compared to observed heterozygosities by the *d* test of Bailey (1974).

Two sample pools were analysed: the first collected between 1987 and 1988, and the other collected between 1992 and 1993, in comparable seasons.

## RESULTS AND DISCUSSION

Table 1 corresponds to the data on the variation found in the Mdh locus analysed in *D. willistoni* populations. It can be observed that urban populations tended to become more polymorphic

TABLE 1

### Allelic frequencies and heterozygosity in the Mdh locus of urban and wild populations of *Drosophila willistoni*

Frecuencias alélicas y heterozigosidad en el locus Mdh de poblaciones urbanas y silvestres de *Drosophila willistoni*

Allele	Sample								
	1987-1988			1992			1993		
	Urban		Wild	Wild	Urban		Wild	Urban	
Redenção	Goethe (+)	Tristeza (++)	Eldorado (+)	Eldorado (+)	P. M. Cardoso (+)	Redenção (+)	Eldorado (+)	P. M. Cardoso (+)	
1.50	-	-	-	-	-	-	-	-	0.0076
1.31	-	-	-	-	-	0.0090	-	-	-
1.29	-	-	-	0.0225	-	-	0.0173	-	-
1.26	-	-	-	-	-	0.0090	0.0690	-	-
1.20	-	-	-	0.0056	0.0800	0.0357	0.0518	0.0278	-
1.14	-	-	-	-	-	-	-	-	0.0152
1.10	-	-	-	-	0.0200	0.0090	-	-	-
1.00	0.9592	1.0000	0.9815	0.9551	0.8000	0.8751	0.8104	0.8834	0.9546
0.94	-	-	-	-	-	0.0090	-	0.0556	-
0.89	-	-	-	-	-	-	0.0345	0.0111	-
0.85	0.0306	-	0.0185	0.0112	0.0800	0.0179	0.0173	-	0.0076
0.82	-	-	-	-	-	0.0268	-	0.0111	0.0151
0.75	-	-	-	-	-	-	-	0.0111	-
0.60	0.0102	-	-	0.0056	-	-	-	-	-
Genes analysed	98	80	54	178	50	112	58	180	132
Obs. het.	0.0408	0.0000	0.0370	0.0899	0.2000	0.1787	0.3104	0.0556	0.0607
Exp. het.	0.0789*	0.0000	0.0363	0.0871**	0.3464***	0.2316***	0.3290***	0.2154***	0.0882*

+ = collected in fruits of *Syagrus romanzoffiana*

++ = collected in fruits of *Averrhoa carambola*

Significant at levels = \*0.05; \*\*0.01; \*\*\*0.001

with time, as seen in the heterozygosis levels that appeared to increase from the first samples (1987-1988) to the later ones (1992-1993). This also seems to be true for the number of alleles detected in this enzymatic system.

The Odh-2 locus (Table 2) clearly showed the same tendency, especially with regard to the comparison of the heterozygosis values, which in the 1987-1988 samples were similar but increased considerably in the urban populations, sampled in 1992 and 1993. On the contrary, the Odh-1 locus (Table 3), only analysed in the years 1992 and 1993, showed inverse tendencies in the number of alleles, in these two samples. In 1992, the urban populations showed more alleles than the wild one, contrary to what occurred in samples of 1993. In both samplings, however, the heterozygosis was higher in the urban populations than in the wild ones.

The  $\alpha$ -Gpdh system, whose functional role in the wing vibration is well known for several winged insects and is also important to the dispersion of coloniser populations, had its variability diminished between the first and the second period of sampling, as can be seen in Table 4. This was true both for the heterozygosity

measurement and for the number of alleles detected. Our interpretation for these findings is that in an initial phase, when the populations were exploiting the new urban environment, heterozygote flies had been favoured, and several alleles had been "tested" in face of the new challenges found. Later, when the populations were already established, the number of possible combinations dropped, a certain tendency to allele fixation emerging. For instance, among the samples of two contiguous and comparable places, as those of O. B. Viana street (1988) and of M. Cardoso square (1992), only one of the alleles initially found showed the tendency of being fixed.

Table 5 shows data of the acid phosphatase (Acph) locus, in which we also observed a tendency for increasing variability between the two periods of sampling, but in this case this phenomenon seems also to occur in wild populations. Some urban populations, however, as those of Redenção Park, that in the first period presented few alleles and low heterozygosity, doubled the number of alleles and showed high heterozygosity in the later samples. The same was verified in the samples from O. B. Viana (1987-1988) and from P. M. Cardoso (1992).

TABLE 2

Allelic frequencies and heterozygosity in the Odh-2 locus of urban and wild populations of *Drosophila willistoni*

Frecuencias alélicas y heterozigosidad en el locus Odh-2 de poblaciones urbanas y silvestres de *Drosophila willistoni*

Allele	Sample											
	1987-1988						1992		1993			
	Urban						Wild		Wild	Urban	Wild	Urban
	Redenção (+)	Goethe (+)	O. B. Viana (+)	Tristeza (++)	C. Batista (+)	J. Botânico (+++)	Eldorado (+)	Eldorado (+)	Eldorado (+)	P. M. Cardoso (+)	Eldorado (+)	P. M. Cardoso (+)
1.14	-	-	-	-	-	-	-	-	-	-	0.0091	-
1.12	0.0263	-	0.0417	0.0323	0.0429	0.0352	0.0336	0.0339	-	0.0435	-	0.0357
1.10	-	-	-	-	-	-	-	-	-	-	-	0.0119
1.00	0.9649	1.0000	0.9583	0.9032	0.9476	0.9648	0.9552	0.9661	0.9791	0.9131	0.9546	0.9047
0.94	-	-	-	-	-	-	-	-	0.0209	-	0.0182	0.0357
0.90	0.0088	-	-	0.0484	0.0095	-	0.0112	-	-	0.0435	0.0091	0.0119
0.81	-	-	-	-	-	-	-	-	-	-	0.0091	-
0.75	-	-	-	0.0161	-	-	-	-	-	-	-	-
Genes	114	66	48	62	210	256	268	118	48	46	110	84
Obs. het. analysed	0.0702	0.0000	0.0833	0.1290	0.1048	0.0547	0.0896	0.0678	0.0417	0.1738	0.0910	0.1190
Exp. het.	0.0682*	0.0000	0.0799	0.1806**	0.1001***	0.0679**	0.0863***	0.0655*	0.0409	0.1625*	0.0880*	0.1787**

+ = collected in fruits of *Syagrus romanzoffiana*

++ = collected in fruits of *Averrhoa carambola*

+++ = collected in fruits of *Butia eriospatha*

Significant at levels = \*0.05; \*\*0.01; \*\*\*0.001

TABLE 3

Allelic frequencies and heterozygosity in the Odh-1 locus of urban and wild populations of *Drosophila willistoni*Frecuencias alélicas y heterozigosis en el locus Odh-1 de poblaciones urbanas y silvestres de *Drosophila willistoni*

Allele	Sample				
	1987-1988		1992		1993
	Urban	Urban	Wild	Urban	Wild
	Redenção (+)	Redenção (+)	Eldorado (+)	P. M. Cardoso (+)	Eldorado (+)
1.75	-	-	-	0.1185	0.0448
1.70	0.0175	-	-	-	0.0373
1.62	0.9825	0.8912	1.0000	0.7368	0.8358
1.55	-	0.0218	-	0.1448	0.0821
1.50	-	0.0870	-	-	-
Genes analysed	114	46	46	76	134
Obs. het.	0.0000	0.1304	0.0000	0.3158	0.1641
Exp. het.	0.0344	0.1977*	0.0000	0.4221***	0.2913***

+ = collected in fruits of *Syagrus romanzoffiana*

Significant at levels = \*0.05; \*\*0.01; \*\*\*0.001

TABLE 4

Allelic frequencies and heterozygosity in the  $\alpha$ -Gpdh locus of urban and wild populations of *Drosophila willistoni*Frecuencias alélicas y heterozigosis en el locus  $\alpha$ -Gpdh de poblaciones urbanas y silvestres de *Drosophila willistoni*

Allele	Sample											
	1987-1988								1992			
	Urban				Wild				Wild	Urban		
	Redenção (+)	Goethe (+)	O. B. Viana (+)	Tristeza (++)	Andaraí (+)	J. Botânico (+++)	C. Batista (+)	Eldorado (+)	Eldorado (+)	Eldorado (+)	Redenção (+)	P.M.Cardoso (+)
1.20	0.0096	-	0.0196	0.0125	-	-	-	0.0109	-	0.0777	-	-
1.17	-	-	-	-	-	-	-	-	-	0.0222	0.0135	-
1.00	0.9904	1.0000	0.9706	0.9750	1.0000	0.9952	1.0000	0.9891	1.0000	0.8667	0.9865	1.0000
0.84	-	-	0.0098	0.0125	-	0.0048	-	-	-	0.0333	-	-
Genes analysed	104	38	102	80	136	210	164	184	134	90	74	86
Obs. het.	0.0192	0.0000	0.0588	0.0500	0.0000	0.0095	0.0000	0.0217	0.0000	0.1554	0.0270	0.0000
Exp. het.	0.0190	0.0000	0.0574	0.0491	0.0000	0.0056	0.0000	0.0216	0.0000	0.2423***	0.0267	0.0000

+ = collected in fruits of *Syagrus romanzoffiana*++ = collected in fruits of *Averrhoa carambola*+++ = collected in fruits of *Butia eriospatha*

Significant at levels = \*0.05; \*\*0.01; \*\*\*0.001

Finally, the Amy system (Table 6), analysed in samples collected in 1993, showed a higher number of alleles and heterozygosity in the urban population in contrast to the wild one.

The conclusions obtained from this analysis of all enzymatic loci comparing urban and wild

populations of *D. willistoni* for sampling periods separated by four years, are as follows. We observed a general tendency of an increase in the number of alleles and/or of the levels of heterozygosity in urban populations, when compared to those from the wild control site. This

TABLE 5

Allelic frequencies and heterozygosity in the Acph locus of urban and wild populations of *Drosophila willistoni*Frecuencias alélicas y heterozigosis en el locus Acph de poblaciones urbanas y silvestres de *Drosophila willistoni*

Allele	Sample											
	1987-1988							1992				
	Urban							Wild		Wild		Urban
Redenção (+)	Goethe (+)	O. B. Viana (+)	Tristeza (++)	J. Botânico (+++)	Andaraí (+)	C. Batista (+)	Eldorado (+)	Eldorado (+)	Eldorado (+)	Redenção (+)	P.M.Cardoso (+)	
1.37	-	-	-	-	-	-	-	-	-	-	-	0.1429
1.20	-	-	-	-	0.0048	-	-	-	-	-	-	-
1.16	0.0263	-	0.0102	0.9474	-	-	0.0342	0.0176	0.0077	0.2427	0.4500	0.2738
1.00	0.9737	1.0000	0.9745	0.0526	0.9904	1.0000	0.9179	0.9471	0.9615	0.6986	0.5000	0.5714
0.87	-	-	-	-	-	-	-	-	-	0.0294	-	0.0119
0.85	-	-	0.0153	-	0.0048	-	0.0479	0.0353	0.0308	-	0.0250	-
0.81	-	-	-	-	-	-	-	-	-	0.0074	0.0250	-
0.75	-	-	-	-	-	-	-	-	-	0.0074	-	-
0.56	-	-	-	-	-	-	-	-	-	0.0074	-	-
Genes analysed	114	38	196	38	208	70	162	170	130	136	40	84
Obs. het.	0.0526	0.0000	0.0510	0.1053	0.0286	0.0000	0.1481	0.0823	0.0769	0.5000	1.0000	0.4762
Exp. het.	0.0512	0.0000	0.0499	0.0997	0.0283	0.0000	0.1399	0.1014*	0.0745	0.4520***	0.5463**	0.5780***

+ = collected in fruits of *Syagrus romanzoffiana*++ = collected in fruits of *Averrhoa carambola*+++ = collected in fruits of *Butia eriospatha*

Significant at levels = \*0.05; \*\*0.01; \*\*\*0.001

is valid for the main proportion of the enzymatic systems evaluated. Considering the short generation period of this species, and the time elapsed between the two sampling periods (1987/1988-1992/1993), we suggest that the increase of genetic variability could be the consequence of the success of *D. willistoni* in becoming established in the new urban environment. The same populations were analysed with respect to their chromosomal polymorphism for paracentric inversions (Valente et al. 1993) and the results clearly showed a significant loss of structural chromosomal variability in urban populations. This finding support the idea of homoselection operating in marginal populations (Carson 1955, 1959). According to this hypothesis, an increase of recombination allowed by the loss of inversions could generate higher allelic variability. This seems to be the case for our populations.

We think that this is the most probable explanation for our findings, given our studies on both urban fly populations for more than fifteen years (Valente & Araújo 1985, Bonorino & Valente 1989, Santos & Valente 1990, Valente et al. 1989, 1993, Bonorino et al. 1993, Regner & Valente 1993, Rohde & Valente 1996, 1997; Valiati & Valente 1996, 1997), and of wild ones (Valente & Araújo 1991, Saavedra et al. 1995).

TABLE 6

Allelic frequencies and heterozygosity in the Amy locus of urban and wild populations of *Drosophila willistoni*Frecuencias alélicas y heterozigosis en el locus Amy de poblaciones urbanas y silvestres de *Drosophila willistoni*

Allele	Sample	
	1993	
	Wild Eldorado (+)	Urban P. M. Cardoso (+)
1.25	-	0.0062
1.12	0.0070	0.0186
1.00	0.8056	0.8088
0.92	0.0209	-
0.89	0.1667	0.1605
0.82	-	0.0062
Genes analysed	144	162
Obs. het.	0.2084	0.2965
Exp. het.	0.3227**	0.3197***

+ = collected in fruits of *Syagrus romanzoffiana*

Significant at levels = \*0.05; \*\*0.01; \*\*\*0.001

During this time, we have observed that the urban environment is unfavorable to wild species (like *D. willistoni* and others). They are poorly represented in the samples emerged from fruits collected in the city, being strongly surpassed by cosmopolitan species as *D. simulans*, *D. hydei*, *D. immigrans*, *D. kikkawai*, and others in smaller scale. These later species clearly present characteristics of being better competitors than the wild studied species (*D. willistoni*, *D. paulistorum*, *D. nebulosa*, *D. cardinoides*, *D. polymorpha*).

With respect to food, we also observed great unpredictability in urbanised places: rotting fruits does not remain available for much time due, in part, to the consumption by other animals, including those associated with human activities (as rats, for instance), or because they are collected by man and swept away from public and/or private places. So, no evidences in the relaxation of the selective forces could be invoked to explain the increase of the genetic variability found in our samples.

#### ACKNOWLEDGMENTS

This study was performed with grants and fellowships of the Brazilian agencies CNPq, FAPERGS, and with grants of FINEP and PROPESQ-UFRGS.

#### LITERATURE CITED

- AYALA FJ, JR POWELL, ML TRACY, CA MOURAO & S PEREZ-SALAS (1972) Enzyme variability in the *Drosophila willistoni* group. IV. Genetic variation in natural populations of *Drosophila willistoni*. *Genetics* 70: 113-139.
- ASHBURNER M (1967) Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. I. Autosomal puffing patterns in a laboratory stock of *D. melanogaster*. *Chromosoma* (Berlin) 27: 398-428.
- BAILEY NTJ (1974) *Statistical methods in biology*. English Universities Press Ltd., London, United Kingdom. 200 pp.
- BONORINO CBC & VLS VALENTE (1989) Studies on wild and urban populations and breeding sites of *Drosophila nebulosa*. *Revista Brasileira de Biologia* 49: 771-776.
- BONORINO CBC, VLS VALENTE & SM CALLEGARI-JACQUES (1993) Urbanization and chromosomal polymorphism of *Drosophila nebulosa*. *Revista Brasileira de Genética* 16: 59-70.
- BORBA CM & M NAPP (1986) Genetic-ecologic correlations in the enzyme variability of *Drosophila willistoni*. *Revista Brasileira de Genética* 9: 593-614.
- BRNCIC D & VLS VALENTE (1978) Dinâmica de comunidades de *Drosophila* que se estabelecem em frutos silvestres no Rio Grande do Sul. *Ciência e Cultura* 30: 1104-1111.
- CARSON HL (1955) The genetic characteristics of marginal populations of *Drosophila*. Cold Spring Harbor Symposium on Quantitative Biology 20: 276-285.
- CARSON HL (1959) Genetic conditions which promote or retard the formation of species. Cold Spring Harbor Symposium on Quantitative Biology 24: 17-105.
- CARSON HL (1965) Chromosomal morphism in geographically widespread species of *Drosophila*. In: Baker HG & GL Stebbins (eds) *The genetics of colonising species*: 508-531. Academic Press, New York, New York.
- CORDEIRO AR & H WINGE (1995) Levels of evolutionary divergence of *Drosophila willistoni* sibling species. In: Levine L (ed) *Genetics of natural populations. The continuing importance of Theodosius Dobzhansky*: 262-280. Columbia University Press, New York, New York.
- DA CUNHA AB & T DOBZHANSKY (1954) A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to the environment. *Evolution* 8: 119-134.
- DA CUNHA AB, T DOBZHANSKY, O PAVLOVSKY & B SPASSKY (1959) Genetics of natural populations. XXVIII. Supplementary data on the chromosome polymorphism in *Drosophila willistoni* in its relation to the environment. *Evolution* 13: 385-404.
- DOBZHANSKY T (1965) "Wild" and "domestic" species of *Drosophila*. In: Baker HG & GL Stebbins (eds) *The genetics of colonizing species*: 533-546. Academic Press, New York, New York.
- GOÑI B, ME MARTÍNEZ & P DAGUER (1997) Studies of two *Drosophila* (Diptera, Drosophilidae) communities from urban Montevideo, Uruguay. *Revista Brasileira de Entomologia* 41: 89-93.
- GOÑI B, ME MARTÍNEZ, VLS VALENTE & CR VILELA (1998) Preliminary data on the *Drosophila* species from Uruguay. *Revista Brasileira de Entomologia* 42: 131-140.
- HÜETTEL RN & GL BUSH (1972) Starch gel electrophoresis of tephritid proteins. A manual of techniques. Group on Fruit Flies. International Biological Programme Press.
- MARQUES EK, M NAPP, H WINGE & AR CORDEIRO (1966) A corn meal, soybean flour, wheat germ medium for *Drosophila*. *Drosophila Information Service* (USA) 41: 187.
- POULIK MD (1957) Starch gel electrophoresis in a discontinuous system of buffers. *Nature* 180: 1477-1479.
- REGNER LP & VLS VALENTE (1993) *Drosophila willistoni* mating activity - urbanization effects and a search for its chromosomal basis. *Evolución Biológica* 7: 327-349.
- RHODE C & VLS VALENTE (1996) Cytological maps and chromosomal polymorphism of *Drosophila polymorpha* and *Drosophila cardinoides*. *Brazilian Journal of Genetics* 19: 27-32.

- ROHDE C & VLS VALENTE (1997) Ecological characteristics of urban populations of *Drosophila polymorpha* Dobzhansky & Pavan and *Drosophila cardinoides* Dobzhansky & Pavan (Diptera, Drosophilidae). *Revista Brasileira de Entomologia* 40: 75-79.
- SAAVEDRA CCR, SM CALLEGARI-JACQUES, MNAPP & VLS VALENTE (1995) A descriptive and analytical study of four neotropical drosophilid communities. *Journal of Zoology, Systematics and Evolutionary Research* 33: 62-74.
- SANTOS RA & VLS VALENTE (1990) On the occurrence of *Drosophila paulistorum* Dobzhansky & Pavan (Diptera, Drosophilidae) in urban environment: ecological and cytological observations. *Evolución Biológica* 4: 253-268.
- SCANDALIOS JG (1969) Genetic control of multiple molecular forms of isozyme in plants: a review. *Biochemical Genetics* 3: 37-79.
- SPASSKY B, RC RICHMOND, S PÉREZ-SALAS, O PAVLOVSKY, CA MOURAO, AS HUNTER, H HOENIGSBERG, T DOBZHANSKY, FJ AYALA (1971) Geography of the sibling species related to *Drosophila willistoni*, and of the semispecies of the *Drosophila paulistorum* complex. *Evolution* 25: 125-143.
- VALENTE VLS & AM ARAUJO (1986) Comments on breeding sites of *Drosophila willistoni* Sturtevant (Diptera, Drosophilidae). *Revista Brasileira de Entomologia* 30: 281-286.
- VALENTE VLS & AM ARAUJO (1991) Ecological aspects of *Drosophila* species in two contrasting environments in Southern Brazil (Diptera, Drosophilidae). *Revista Brasileira de Entomologia* 35: 237-253.
- VALENTE VLS, A RUSZCZYK, RA SANTOS, CBC BONORINO, BEP BRUM, LP REGNER & NB MORALES (1989) Genetic and ecological studies on urban and marginal populations of *Drosophila* in the South of Brazil. *Evolución Biológica* 3: 19-35.
- VALENTE VLS, A RUSZCZYK & RA SANTOS (1993) Chromosomal polymorphism in urban *Drosophila willistoni*. *Revista Brasileira de Genética* 16: 307-317.
- VALIATI VH & VLS VALENTE (1996) Observations on ecological parameters of urban populations of *Drosophila paulistorum* Dobzhansky & Pavan (Diptera, Drosophilidae). *Revista Brasileira de Entomologia* 40: 225-231.
- VALIATI, VH & VLS VALENTE (1997) Chromosomal polymorphism in urban populations of *Drosophila paulistorum*. *Brazilian Journal of Genetics* 20: 567-581.

*Invited Editors: R. Godoy-Herrera and G. Gajardo*

*Received April 21, 2000; accepted September 18, 2000*