

LONG-TERM CHANGES AND SEASONAL FLUCTUATIONS IN THE CHROMOSOMAL POLYMORPHISM OF CHILEAN POPULATIONS OF *Drosophila immigrans*

Danko Brncic

ABSTRACT

Chilean populations of *Drosophila immigrans* are polymorphic for three paracentric inversions in the second chromosome designated as A, B and C. The other chromosomes are homosequential. In the last 35 years, substantial changes have been detected in the distribution or frequencies of two of the inversions. Inversion C, originally restricted to a local area near Valdivia (Lat. 39°41'S) in the 1950'S, has extended its distribution over the country from Lat. 29°54'S to Lat. 41°28'S. Inversion B, the only inversion present in all the populations examined, exhibits significant seasonal fluctuations in frequency. Inversion A, present in the Central and Southern regions of Chile, shows neither changes in seasonal frequencies nor in its distribution in Chile. It can be concluded that microevolutionary changes in *D. immigrans*, and probably in other species of the genus, are faster than formerly postulated.

INTRODUCTION

The cosmopolitan species *Drosophila immigrans* is one of the most common species of *Drosophila* throughout the Chilean territory, from Arica in the Peruvian border (Lat. 18°29'S) as far as Punta Arenas in the Strait of Magellan (Lat. 53°09'S). Although this species can be observed throughout the year, the populations exhibit a peak in Spring. During this season, populations of *D. immigrans* coexist, at least in the Central part of Chile, with endemic species such as *D. pavani*, with the recently introduced *D. subobscura* and with some other cosmopolitan species. Like other species of this genus,

including many cosmopolitan ones (see Sperlich and Pfriem, 1986 and Ashburner, 1989), natural populations of *D. immigrans* are polymorphic due to paracentric inversions in the chromosomes. In Chile, the species present three different inversions (Brncic, 1955). They are the same as those observed by Richmond and Dobzhansky (1968) in Hawaii and in other parts of the world (see Ashburner, 1989). A previous analysis has shown clear interpopulational differences in the polymorphisms which occur in this species in Chile (Brncic, 1955). Since that study, no systematic analysis of the chromosomal composition of Chilean populations has been made.

The main purpose of the present report is to give an account of the evolution of the chromosomal polymorphisms occurring in *D. immigrans* since the early studies done in the of 1950'S. During this period, many changes occurred in the *Drosophila* communities in Chile, including the introduction of a new species, *D. subobscura* (Brncic and Budnik, 1980). Moreover, the environment in which the species live has been modified by increasing urbanization, industrialization, new agricultural practices and other human activities. In addition to the existence of long-term variations in chromosomal polymorphism, it has been observed that some of the chromosomal morphs in the natural populations suffer seasonal variations that merit discussion.

MATERIAL AND METHODS

Demographic studies of *D. immigrans* were done from 1953 to 1990. Flies were collected from fermented banana baits located in a small orchard in the district of La Florida (Santiago). The orchard contained ornamental plants, a few fruit-trees (plums, apricots, figs, mulberries, medlar-trees) and some native vegetation. In 1953, the La Florida district was basically a suburb with a human population of 25.000 inhabitants. It was primarily dedicated to farming and horticultural activities. Since then, the district has become more urbanized and it now constitutes a prosperous part of the Metropolitan area of Santiago City with a population near 400,000. Species seasonality has been analyzed through monthly collections from the above mentioned area from 1984 to 1990.

In order to analyze the seasonal fluctuations of chromosomal polymorphism, samples of *D. immigrans* were collected in the Springs of 1988 and 1989 and in the Autumns of 1989 and 1990. Wild inseminated females were placed individually in vials with *Drosophila* food media enriched with live yeast and allowed to oviposit. In order to study the banding patterns of giant chromosomes, the salivary glands of third instar larvae of the progeny were used and prepared by means of the aceto-lactic-orcein rapid squash method. For quantitative analysis, only one larva per vial was recorded. Long-term variations in both the distribution of the species and the different gene arrangements throughout the country were derived by comparing data from the early records obtained in the period of 1950-1960 with that obtained in recent years.

RESULTS

D. immigrans one of the species present in Central Chile throughout the year, exhibited a population expansion during the Spring (Figure 1). An analysis of samples of *Drosophila* taken in Central Chile from 1953 to 1990 revealed that there was general tendency towards a decrease in the relative frequency of *D. immigrans* between 1953 and 1987, followed by an increase in 1988 and 1989. This tendency is illustrated in Figure 2. The Spring frequencies of *D. immigrans* are shown together with those of *D. subobscura*, a species that reaches its maximum peak in the same months as *D. immigrans*.

Another interesting fact is the recent finding of *D. immigrans* in the City of Punta Arenas in the Strait of Magellan (Lat. 53°10'S). According to previous records the Southern limit of the species was located at about Lat. 44°S. So, *D. immigrans* has become one of the southernmost distributed species of the genus.

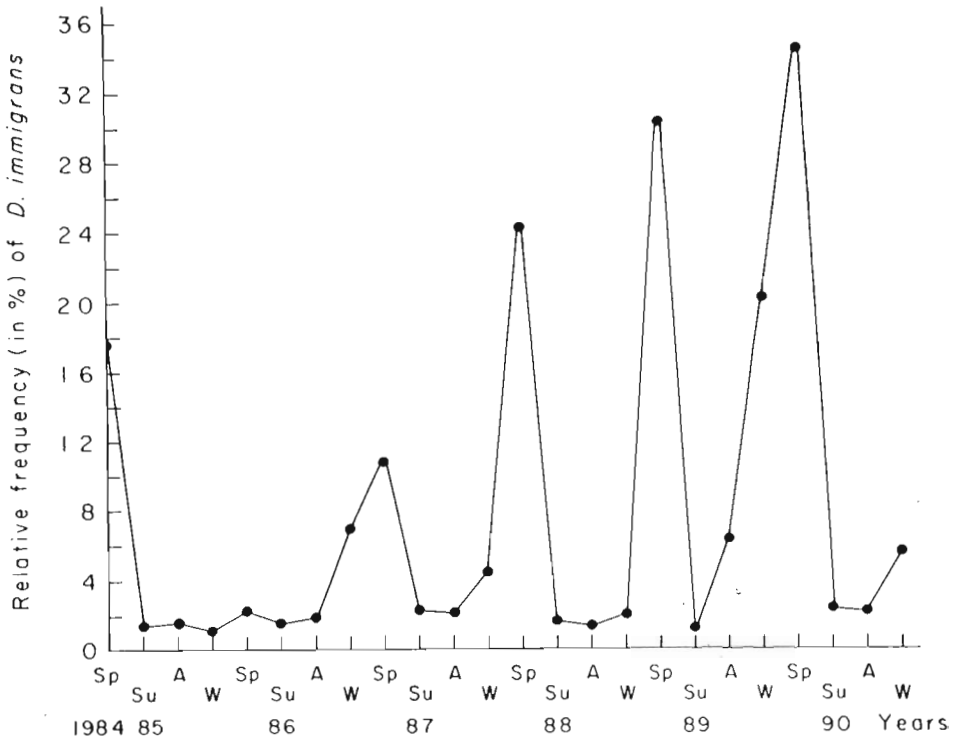


Figure 1 - Seasonal variations in the relative frequency of *D. immigrans* in "La Florida" (Santiago) analyzed from 1984 to 1990 (Sp = Spring; Su = Summer; A = Autumn, W = Winter). (Samples sizes range between 734 to 3593).

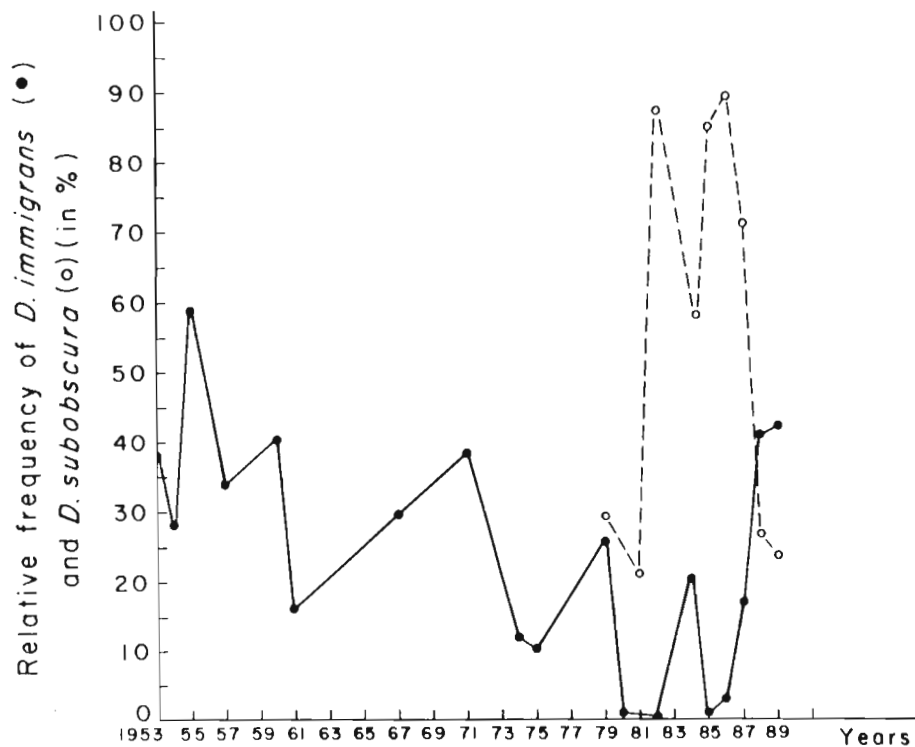


Figure 2 - Relative frequencies of *D. immigrans* and *D. subobscura* collected in the spring months in "La Florida" (Santiago) from 1953 to 1990. (Sample size mean = 584,50 ± 293,14).

The karyotype of *D. immigrans* is composed of one pair of sexual chromosomes (Chr.1) - the X, a mediumlong rod, and the Y, a short V-shaped chromosome; one pair of V-shaped chromosomes (Chr.2); one pair of long rods (Chr.3) and one pair of short rods (Chr.4) (Le Calvez, 1953). As previously reported (Brcic, 1955), the Chilean populations of *D. immigrans* are polymorphic for three gene arrangements in the metacentric second chromosome: a) Inversion A, found in the middle part of the left arm; b) Inversion B, a smaller subterminal inversion in the right arm of the same chromosome; and c) Inversion C, a small inversion located in the proximal part of the right arm. These three inversions have also been found in Hawaii (Richmond and Dobzhansky, 1968; Paik and Sung, 1974) and India (Parshad and Arora, 1971), while A and B have been observed in Japan (Toyofuku, 1961) and inversion A was discovered in Brazil (Freire Maia *et al.*, 1953). Other inversions cited in the literature (Sperlich and Pfriem, 1986) have never been observed in Chile. Photomicrographs showing the inversion heterozygotes found in Chile are presented in Figure 3.

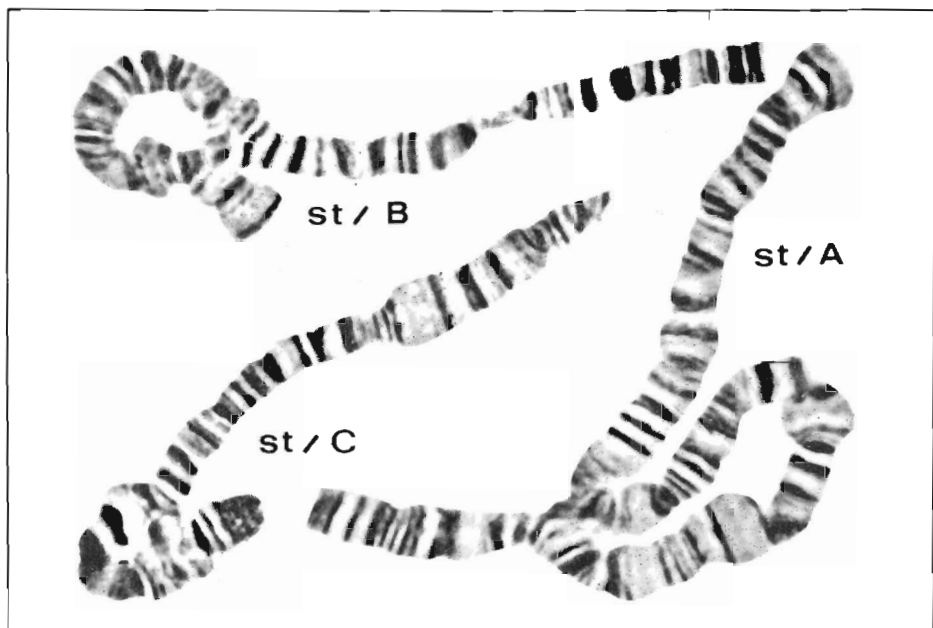


Figure 3 - Photomicrographs of the heterozygous inversions A, B and C of *D. immigrans*.

While comparing the old records of distribution of the heterozygotes for the different chromosomal arrangements in *D. immigrans* (Brcic, 1955) with the recent records (Table I), it was observed that inversions A and B maintained practically the same distribution. These two inversions have been found in the newly collected samples from Punta Arenas also. By contrast, inversion C, previously observed only in a local population near Valdivia (Lat. $39^{\circ}41'S$), has extended its range from Lat. $29^{\circ}54'S$ to Lat. $41^{\circ}28'S$. This fact deserves further comment.

Table II shows the frequencies of heterozygous inversions found in two successive years during the Spring and Autumn in La Florida (Santiago). The homozygotes for standard and inverted gene arrangements were arranged in one column because the method employed and the knowledge available about the banding sequences did not permit easy identification. The frequency of heterozygotes increased significantly during the Autumn months (Table III). This frequency change seems to be due to an increase in heterozygotes with inversion B. According to the Chi-square homogeneity test, the differences between Spring and Autumn samples are highly significant (Table IV). Apparently, inversion A does not undergo seasonal fluctuations as no significant differences were observed between the Spring and Autumn samples in the two successive years of observation (Table V). The fact that there was a low frequency of heterozygotes with

inversion C prevents us from further studying any seasonal fluctuations for this particular inversion. The frequencies of both A and B heterozygous inversions observed in the Autumn of 1955 from the same locality were 18.5 (Brcic, 1955). These values are not too different from those observed in the 1989-1990 samples.

Table I - Localities in Chile analyzed for chromosomal polymorphism (A - heterozygous inversion A in the 2-L chromosome; B and C - heterozygous inversions B and C in the 2-R chromosome).

Locality		Latitude	1953-1960	1980-1990
Arica	(*)	18°29'S	B	**
Copiapó		27°22'S	**	BC
La Serena	(*)	29°54'S	B	B
Paihuano	(*)	30°02'S	B	**
Ovalle	(*)	30°57'S	B	B
Los Vilos		31°54'S	**	B
La Florida (Stgo)	(*)	33°30'S	B	ABC
Vizcachas (Stgo)		33°35'S	**	ABC
San Vicente	(*)	34°27'S	AB	ABC
Chillán		36°37'S	AB	ABC
Salto del Laja		37°10'S	AB	ABC
Valdivia	(*)	39°48'S	ABC	ABC
Osorno		40°35'S	AB	ABC
Puyehue		40°42'S	**	ABC
Rupanco		40°45'S	AB	ABC
Puerto Octay		41°00'S	**	ABC
Ensenada	(*)	41°12'S	AB	**
Puerto Varas	(*)	41°20'S	AB	**
Puerto Montt	(*)	41°28'S	**	ABC
Punta Arenas		53°09'S	**	AB

* Localities reported in Brcic (1955).

** Not sampled.

Table II - Frequency (in %) of heterozygous inversions in Spring and Autumn for two successive years.

		1988-89		1989-90	
		Spring	Autumn	Spring	Autumn
N		100	100	100	79
Total Hom.		71	59	67	49.37
Total Het.		29	41	33	50.63
	A	20	17	19	20.25
	B	6	14	9	21.52
	A + B	1	6	1	3.80
	C	2	4	4	5.06
Total	A	21	23	20	24.05
Total	B	7	20	10	25.32

Table III - Statistical analysis of the differences in total heterozygous inversions between seasons and years (2 x 2 contingency tables).

	Total	Spring	Autumn	Chi ²	P (1 df)
Total Het.	143/379	62/200	81/179	8.17	< 0.001
1988-89	70/200	29/100	41/100	3.16	0.08-0.07
1989-90	73/179	33/100	40/79	5.68	0.02-0.01
Chi ²	1.34	0.37	1.65		
P (1 df)	0.30-0.20	0.60-0.50	0.30-0.20		

Table IV - Statistical analysis of the differences in heterozygotes for inversion B between seasons and years (2 x 2 contingency tables).

	Total	Spring	Autumn	Chi ²	P (1 df)
Total Het.	57/379	17/200	40/179	14.17	< 0.001
1988-89	27/200	7/100	20/100	7.24	< 0.01
1989-90	30/179	10/100	20/79	7.42	< 0.01
Chi ²	0.79	0.58	0.72		
P (1 df)	0.40-0.30	0.50-0.40	0.40-0.30		

Table V - Statistical analysis of the differences in heterozygotes for inversion A between seasons and years (2 x 2 contingency tables).

	Total	Spring	Autumn	Chi ²	P (1 df)
Total Het.	83/379	41/200	42/179	0.49	0.50-0.40
1988-89	44/200	21/100	23/100	0.12	0.80-0.70
1989-90	39/179	20/100	19/79	0.42	0.60-0.50
Chi ²	0.002	0.03	0.03		
P (1 df)	> 0.95	> 0.95	> 0.95		

DISCUSSION

Literature on long-term studies of chromosomal polymorphism in natural populations of *Drosophila* is scarce. The studies of Dobzhansky (1958), Dobzhansky *et al.* (1964, 1966) and Anderson *et al.* (1975), summarizing three decades of research in *D. pseudoobscura*, are exceptions. These authors observed substantial long-term frequency changes in certain inversion polymorphisms occurring in the Western and South-Western regions of the United States. These changes, which occurred in the same direction but with a different speed, were hypothetically attributed to modifications in the environment due to pesticides and other synanthropic factors. In the European species *D. subobscura*, long-term comparisons of chromosomal polymorphism in natural populations near Zürich revealed significant differences during the period of 1963 to 1987 (Gosteli, 1990).

In the Chilean populations of *D. immigrans*, the most striking change that has occurred during the last 35 years is the change in inversion C. It has progressed from being a local morph to one that it is now widely distributed throughout much of the North and South-Central regions of the country. It appears that the other two inversions, A and B, did not change in distribution. Although it is difficult to assess the causes of the observed changes, factors associated with human activities could be involved. During the last decades the country has undergone drastic changes due to urbanization and new agricultural practices. These changes have modified not only the environment, but also the possibilities of insect passive transportation. Nevertheless, there are other possible explanations for the changes in the distribution of inversions. It must be born in mind that the chromosomal polymorphism occurring in *Drosophila* populations is maintained by balancing selection over the coadapted gene complexes located in the inverted section

of the chromosomes. There exists some evidence that the integrated gene contents at the inversions could be altered by mutation and recombination, and thereby cause a change in their Darwinian fitness (Dobzhansky, 1971). However, at present, proof of this possibility must wait for further knowledge on the evolution of the genetic systems in *Drosophila*. In considering both the reported long-term evolutionary changes experienced by some North-American populations of *D. pseudoobscura*, the ones observed in Chilean populations of *D. immigrans* and the rapid changes summarized by Prevosti *et al.* (1990) in the colonized populations of *D. subobscura* also in Chile, it could be concluded that microevolutionary processes in *Drosophila* occur faster than was previously suspected.

Not all Chilean species experience long-term changes such as those described for *D. immigrans*. The extreme case is represented by *D. pavani*, a typical endemic species in which the chromosomal polymorphism has not suffered any modification in the last 35 years, despite environmental degradation and the drastic demographic changes observed in the species. *D. pavani* was at one time the dominant species, however over time it has become extremely rare in the area. The high stability of the chromosomal polymorphism in this species has been attributed to the strong heterotic properties of the inversion heterozygotes (Brcic, 1989).

In contrast to the long-term changes found in inversion C, inversion B shows yearly seasonal cyclic frequency changes. As in other polymorphic species, including the cosmopolitan *D. melanogaster* and *D. funebris*, and many endemic species (Sperlich and Pfriem, 1986; Ashburner, 1989), it is most probable that the seasonal changes observed in inversion frequencies is due to natural selection. Nevertheless, the reasons why some chromosomal morphs are favored in Spring and others in Autumn remain to be investigated. Many differences exist in the population dynamics of the species, including changes in abundance and density at different seasons of the year. It is known that under laboratory conditions, in species like *D. pseudoobscura* (Birch, 1955) and *D. pavani* (Budnik *et al.*, 1971), crowding and other density dependent factors can modify the frequency of certain inversions. But the seasons of Spring and Autumn represent environmental variables that also affect many density independent factors, such as temperature and humidity, that could be responsible for the Darwinian fitness of the carriers of the different gene arrangements. Laboratory work carried out in the flower-breeding Chilean species *D. flavopilosa*, whose populations extend over the whole year, revealed that the temperature at which larvae develop is the main factor for seasonal frequency changes in the inversions present in the species (Brcic, 1983).

Finally, it seems clear that long-term changes or seasonal fluctuations observed in the chromosomal polymorphism of *Drosophila* occur just in some species and with respect to some inversions only. These variations could be interpreted as different genetically determined adaptive strategies.

ACKNOWLEDGMENTS

I am grateful to Professor Remigio López-Solis for his valuable comments on the manuscript.

This work has been supported by Grant B 2308-8945 from the University of Chile and Grant 91-0967 from Fondo Nacional de Ciencia (FONDECYT) - Chile.

RESUMO

Populações chilenas de *Drosophila immigrans* são polimórficas para três inversões paracêntricas no segundo cromossomo, denominadas de A, B e C. Os outros cromossomos são homossequenciais. Nos últimos 35 anos, alterações substanciais foram detectadas na distribuição das frequências de duas destas inversões. A inversão C, originariamente restrita a uma área local perto de Valdivia (lat. 39° 41'S) na década de 1950, estendeu sua distribuição no país da lat. 29° 54'S até a lat. 41° 28'S. A inversão B, a única presente na população analisada, apresenta flutuações sazonais significativas de frequência. A inversão A, que está presente nas regiões Central e Sul do Chile, não apresenta modificações sazonais de frequência nem de distribuição no Chile. Pode-se concluir que as modificações microevolutivas de *D. immigrans*, e provavelmente de outras espécies do gênero, são mais rápidas do que postulado anteriormente.

REFERENCES

- Anderson, W. Dobzhansky, Th., Pavlovsky, O., Powell, J. and Yardley, D. (1975). Genetics of natural populations. XLII. Three decades of genetic change in *Drosophila pseudoobscura*. *Evolution* 24: 24-36.
- Ashburner, M. (1989). *Drosophila. A Laboratory Handbook*. Cold Spring Harbor Lab. Press, New York.
- Birch, L.C. (1955). Selection in *Drosophila pseudoobscura* in relation to crowding. *Evolution* 9: 389-399.
- Brcic, D. (1955). Chromosomal variation in Chilean populations of *Drosophila immigrans*. *J. Hered.* 46: 59-63.
- Brcic, D. (1983). Ecology of flower breeding *Drosophila*. In: *The Genetics and Biology of Drosophila* (Ashburner M., Carson, H.C. and Thompson, J.N., eds.). Academic Press Inc. London Vol. 3d pp. 333-382.
- Brcic, D. (1989). Long-term changes in the population ecology of *Drosophila pavani* not followed by changes in the chromosomal polymorphism. *Brazil. J. Genet.* 12: 525-533.
- Brcic, D. and Budnik, M. (1980). Colonization of *Drosophila subobscura* Collin in Chile. *Drosoph. Inf. Serv.* 55: 20.
- Budnik, M., Brcic, D. and Koref-Santibañez, S. (1971). The effect of crowding on chromosomal polymorphism in *Drosophila pavani*. *Evolution* 25: 410-419.
- Dobzhansky, Th. (1958). Genetics of natural populations. XXVIII. The genetic changes in populations of *Drosophila pseudoobscura* in the American Southwest. *Evolution* 12: 385-401.
- Dobzhansky, Th. (1971). Evolutionary oscillations in *Drosophila pseudoobscura*. In: *Ecological Genetics and Evolution* (Creed, R., ed.). Blackell, Oxford pp. 109-133.

- Dobzhansky, Th., Anderson, W., Pavlovsky, O., Spassky, B. and Wills, C.J. (1964). Genetics of natural populations XXXV. A progress report of genetic changes in populations of *Drosophila pseudoobscura* in the American Southwest. *Evolution* 18: 164-176.
- Dobzhansky, Th., Anderson, W. and Pavlovsky, O. (1966). Genetics of natural populations. XXXVIII. Continuity and changes in populations of *Drosophila pseudoobscura* in Western United States. *Evolution* 20: 418-427.
- Freire-Maia, N., Zanardini, I.F. and Freire-Maia, A. (1953). Chromosome variation in *Drosophila immigrans*. *Dusenía*(Brasil) 4: 303-311.
- Le Calvez, J. (1953). Carte cytologique des chromosomes geant des glandes salivaires de *Drosophila immigrans* Sturt. *Chromosoma* 6: 170-174.
- Gosteli, M. (1990). Chromosomal polymorphism in natural populations of *Drosophila subobscura* near Zürich, Switzerland: a contribution to long-term comparisons. *Genetica* 81: 238-240.
- Paik, Y.K. and Sung, K.C. (1974). Variation in chromosomal polymorphism in Hawaiian populations of *Drosophila immigrans*. *Jpn. J. Genet.* 65: 238-240.
- Prevosti, A., Serra, L., Segarra, C., Aguadé, M., Ribó, G. and Monclús, M. (1990). Clines of chromosomal arrangements of *Drosophila subobscura* in South America evolve closer to Old World patterns. *Evolution* 44: 218-221.
- Parshad, R. and Arora, V.K. (1971). Polytene chromosomes of *Drosophila immigrans*. Sturtevant 1921. *Rev. Bull. Panjab Univ. Sci.* 22: 19-22.
- Richmond, R. and Dobzhansky, Th. (1968). Chromosomal polymorphism in populations of *Drosophila immigrans* on the island of Maui, Hawaii. *Studies in Genetics IV*. The Univ. of Texas Publ. 6818: 381-386.
- Sperlich, D. and Pfriend, P. (1986). Chromosomal polymorphism in natural and experimental populations. In: *The Genetics and Biology of Drosophila* (Ashburner, M., Carson, H.C. and Thompson, J.N., eds.). Academic Press Inc. London. Vol. 3e pp. 257-309.
- Toyofuku, Y. (1961). Chromosomal polymorphism found in natural populations of *Drosophila immigrans*. *Jpn. J. Genet.* 36: 32-37.

(Received June 27, 1991)