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LONG-TERM CHANGES IN THE POPULATION ECOLOGY OF *Drosophila pavani* NOT FOLLOWED BY CHANGES IN THE CHROMOSOMAL POLYMORPHISM

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ABSTRACT

During the last 35 years drastic changes in the populations of the native species *Drosophila pavani* have been detected in suburban localities in Central Chile. Before 1979, the species was the most abundant in the area but now it is an extremely rare one. This change could be attributed to environment degradation by increasing urbanization and coincides with the successful introduction of species such as the paleartic *D. subobscura*. A comparison of the inversion polymorphism existing in the natural populations of *D. pavani*, in samples taken in the same place and season in 1955, 1961, 1971, 1980 and 1984-87, shows no differences in the frequency of the different gene arrangements. The extreme stability of the polymorphism in the species contrasts with that found in other species for which changes in the environment are followed by genetic changes. The condition found in *D. pavani* could be interpreted as an expression of the strong heterotic properties of the inversion heterozygotes.

INTRODUCTION

Drosophila pavani is a neotropical species, well represented in the central part of Chile and in a small part of the Andean region of Argentina (Brncic, 1973). During the last years, there has been a general tendency towards a decrease in the relative frequency of the species in *drosophila* collections and, in many localities, *D. pavani* has become rare or absent after being the most abundant and dominant species (Brncic

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and Budnik, 1988). The changes in the species communities could depend on many factors, not easy to evaluate and individualize. For instance, in the central region of Chile, the decrease in abundance of some native species like *D. pavani* has not only coincided with the degradation of the environment by increasing urbanization, industrialization, intensive farming and other anthropogenic factors, but also with the successful recent introduction of invasive species such as the paleartic species *D. subobscura*.

Natural populations of *D. pavani* are polymorphic for gene arrangements due to the presence of paracentric inversions. In the distrit of "Bellavista" (La Florida), now in the metropolitan area of Santiago, samples of *D. pavani* have been obtained periodically since 1954, and many of them were submitted to cytogenetic analysis. Literature on long and continued studies of polymorphism in nature is scarce. Exceptions include works on lepidoptera (summarized by Ford in 1971), and the studies of Dobzhansky (1958), Dobzhansky *et al.* (1964, 1966), and Anderson *et al.* (1975) which give an account of three decades of genetic changes in *D. pseudoobscura*. We report here the results of 35 years of studies on chromosomal polymorphisms in *D. pavani* in a distrit that has been submitted to drastic changes in both the physical environment and the structure of the drosophila community.

MATERIALS AND METHODS

Flies were collected in the distrit of "Bellavista" (La Florida - Santiago), in a small orchard where most vegetation are ornamental plants, a few old fruit trees and some native vegetation. When the first collections were made in 1953, the distrit was mostly suburban with large orchards, some farming and a human population estimated at 25,000. Now, a fully urban part of the metropolitan area of Santiago City with increasing urbanization and a human population of over 300,000 has emerged. The population of the metropolitan area of Santiago is now about 5,000,000. Periodical collections were performed during the last 35 years by net-sweeping over fermenting banana baits. Table I shows the collections made in the spring season (October to early December) which coincided with the maximum peak of *D. pavani* populations.

Cytogenetic analysis of *D. pavani* samples, obtained on the dates indicated in Figure 1, were performed both on females and males. Wild inseminated females were placed individually in vials with the usual drosophila food media, allowed to produce offspring and the salivary glands of third instar larvae were examined in order to study the banding patterns of the chromosomes. Slides were prepared by means of the aceto-lactic-orcein rapid squash method. For quantitative analysis, only one larvae per vial was recorded. Collected males were individually mated to virgin females homozygous for the "Standard" gene arrangements. The salivary gland cells of eight larvae from each cross were examined in order to determine the chromosomal orders of the wild male. Observation of eight larvae renders the chance error negligible.

Table I - Relative frequencies (in %) of *D. pavani, D. subobscura* and other species collected since 1953 to 1987 in a suburban zone of central Chile (La Florida-Santiago).

Date	Flies collected	*D. pavani*	*D. subobscura*	Cosmopolitan species (1)	Subcosmopolitan and widespread species (2)	Endemic species (3)
Nov. 1953	520	41.15		58.66	0.19	–
Nov. 1954	413	60.05		39.23	–	0.72
Nov. 1955	1.397	32.29		65.13	–	2.58
Oct. 1957	497	46.08		47.89	0.20	5.83
Nov. 1960	522	41.38		50.58	1.14	6.90
Oct. 1961	708	55.79		30.93	–	13.28
Nov. 1967	297	20.53		75.76	0.67	3.71
Oct. 1971	992	13.81		56.26	–	29.93
Oct. 1974	238	42.44		28.99	–	28.57
Dec. 1975	710	27.04		71.41	–	1.55
Dec. 1979	162	12.34	29.63	45.68	2.47	9.88
Oct. 1980	526	0.95	16.35	2.47	–	80.23
Oct. 1982	361	–	87.53	5.00	0.83	6.64
Nov. 1984	325	4.00	58.15	37.23	–	0.62
Nov. 1985	720	3.61	85.00	10.00	–	1.39
Nov. 1986	599	0.83	89.65	5.79	–	3.73
Nov. 1987	1.099	0.09	71.34	27.11	–	1.46

1) *D. busckii, D. immigrans, D. funebris, D. hydei, D. melanogaster, D. repleta, D. simulans.*

2) *D. buzzatii, D. mercatorum, D. nigricrucia, D. virilis.*

3) *D. araucana, Scaptomiza denticauda, S. intermedia, S. melancholica, S. multispinosa.*

The populations of *D. pavani* are polymorphic for the genetic arrangements in chromosomes 2 and 4 (Brncic, 1956, 1973). In chromosome 2, besides the "Standard" gene arrangement, there exists a second one formed by two inversions, one included in the other, never observed separately (Inv. II-A + B). In the right arm of chromosome 4, there is a complex gene sequency that differs from the "Standard" by three overlapping inversions always found together (Inv. IVR-A + B + C) and, in the left arm of the same chromosome, besides the "Standard" gene arrangement, there is a second one also consisting of three always clustered, overlapping inversions (Inv. IVL-A + B + C) (Brncic, 1973).

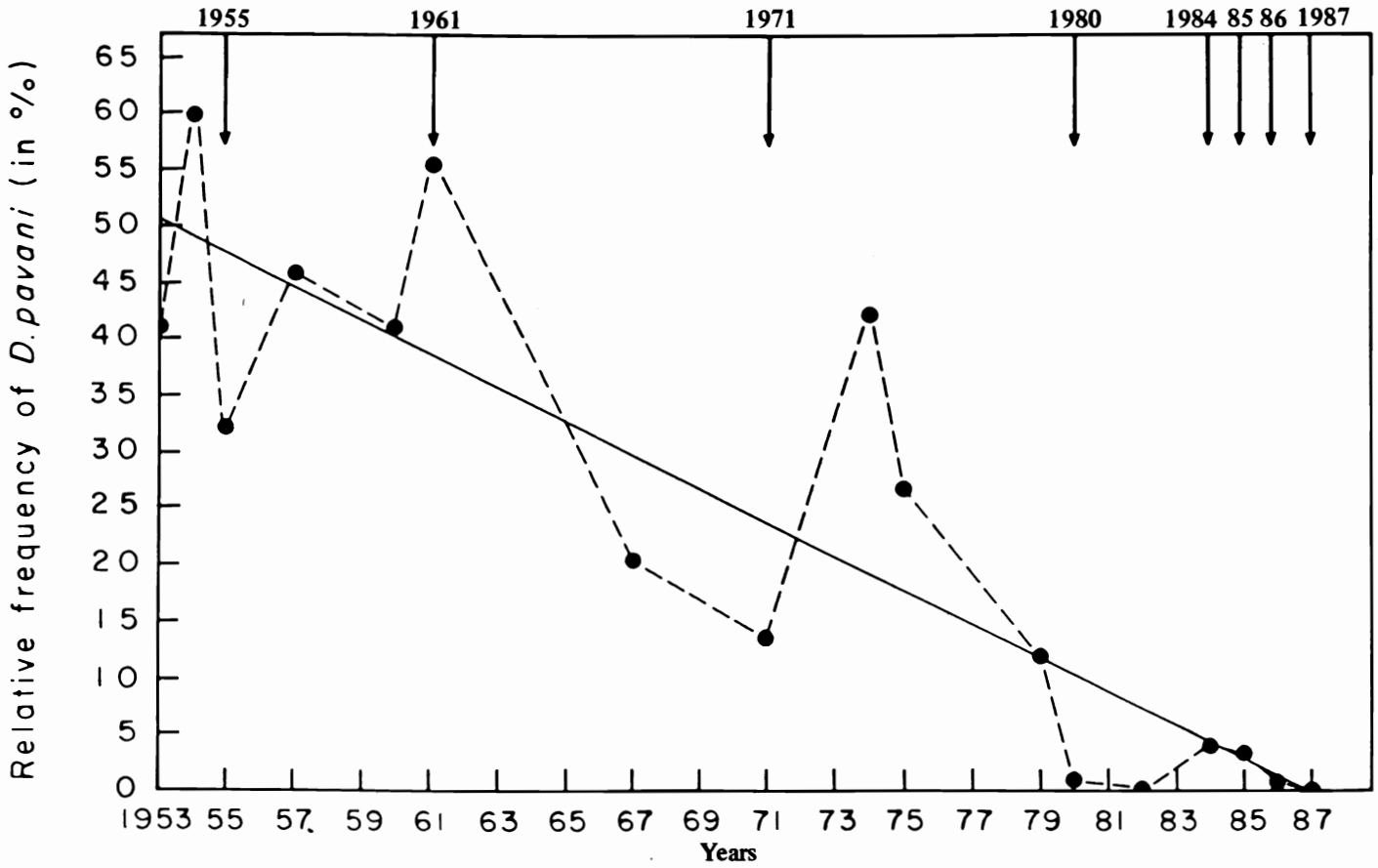

Figure 1 - Relative frequency (%) of *D. pavani* in the locality of "Bellavista" (La Florida) located in the metropolitan area of Santiago, in spring collections performed from 1953 to 1987. The arrows indicate the dates of cytogenetic analysis.

RESULTS AND DISCUSSION

Table I summarizes the results of 17 samples obtained during spring in "Bellavista" (La Florida) from 1953 to 1987. Eighteen species clustered in three categories, have been recorded in the area: (1) *cosmopolitan* (7 species); (2) *subcosmopolitan* and *widespread* (5 species including *D. subobscura*) and (3) *endemic* for Central Chile (6 species including *D. pavani*). Only the relative frequencies of *D. pavani* and *D. subobscura* are given in separate columns. Table I and Figure 1 show that the frequency of *D. pavani* dropped steadily since the first collection records. The species was the most conspicuous and abundant in the area before 1979 but now has become rare. The drop of *D. pavani* coincided with the successful introduction of the paleartic species *D. subobscura* that now is the dominant one. It is known that through resource competition, newly introduced species may interact negatively with the species of the local community thus explaining the population decrease of *D. pavani*. Although difficult to prove, it could be postulated that successful introduction of new species like *D. subobscura* and the decline of others could result from more systematic changes such as environmental degradation due to increasing urbanization. Studies in progress indicate that *D. pavani* has decreased in frequency in practically all of its distributional area, although many of the samples were obtained in completely wild environments.

The frequency data of the different gene arrangements found in "La Florida" district by the examination of wild female offspring and wild male crosses are summarized in Table II - IV. The data corresponding to the 1955 and 1971 samples have been already published (Brncic, 1973) and those of 1984 to 87, were clustered in one column since they deal with the analysis of a very small number of flies obtained in the collections. With regard to the gene arrangements in chromosome 2, it was difficult to make precise identification of "Standard" and "inverted" homozygotes. Therefore, Table II only gives the frequencies of homo- and heterozygotes. A Chi-square test indicates that no substantial changes in the frequencies have been produced since 1955 to 1987. With respect to the gene sequencies in the right and left arms of chromosome 4, Table III indicates that no significant changes in polymorphism have taken place during the 33 year period of observation. Table IV shows that, with only one exception, the observed number of homo- and heterozygotes for the gene arrangements in chromosome 4 are in agreement with the expected values, according to the Hardy-Weinberg distribution. In most samples, there is a slight excess in the observed number of heterozygotes with respect to the expectations. This tendency persisted through all the study period.

Long-term frequency changes of inversion polymorphisms have been observed in *D. pseudoobscura* in the Western and South-Western United States through three decades of observations (Dobzhansky *et al.*, 1964, 1966, Anderson *et al.*, 1975). In

Table II - Number (N) and frequencies (in percent) of homokaryotypes and heterokaryotypes in chromosome 2 of *D. pavani*.

Year of collection	Flies tested	Homokaryotypes		Heterokaryotypes	
		No.	%	No.	%
1955	159	86	54.09	73	45.91
1961	168	101	60.12	67	39.88
1971	100	54	54.00	46	46.00
1980	100	52	52.00	48	48.00
1984-87	61	32	52.45	29	47.55

$\chi^2 = 2.38$; P (df 4) = 0.80 - 0.70.

Table III - Number (N) and frequencies (in percent) of standard and inverted (inv.) gene arrangements in the right (R) and left (L) arms of chromosome 4 of *D. pavani*.

Year of collection	Chromosomes tested	Chromosome 4-R				Chromosome 4-L			
		Standard inversion				Standard inversion			
		No.	%	No.	%	No.	%	No.	%
1955	318	136	42.8	182	57.2	93	29.2	225	70.8
1961	336	135	40.2	201	59.8	89	26.5	247	73.5
1971	200	78	39.0	122	61.0	69	34.5	131	65.5
1980	200	82	41.0	118	59.0	49	24.5	151	75.5
1984-87	122	50	40.9	72	59.1	42	34.4	80	65.6

$\chi^2 = 0.83$; P(4df) = 0.90-0.80 $\chi^2 = 7.67$; P(4df) = 0.10-0.08

most localities, the changes occurred in the same direction, although in some places they seem to be faster. These changes were tentatively attributed to alterations in the environment such as the massive use of pesticides. It was suggested that some chromosomal arrangements were better adapted to the new conditions. The cytogenetic studies in *D. pavani* showing no changes in the chromosomal polymorphism, are in striking contrast to the findings in *D. pseudoobscura*. It is likey that *D. pavani* populations have effectively changed during the period due to the increasing urbaniza-

Table IV - Observed (O) and expected number (E), according to Hardy-Weinberg of homo- and heterozygotes for the gene arrangements in the right (R) and left (L) arms of chromosome 4 of *D. pavani*.

Year of collection	Flies tested		Chromosome 4-R				Chromosome 4-L			
			ST/ST	ST/INV	INV/INV	χ^2	ST/ST	ST/INV	INV/INV	χ^2
1955	159	O	24	88	47		14	65	80	
		E	29.07	77.83	52.09	2.71	13.60	65.81	79.59	0.02
1961	168	O	24	87	57		15	59	94	
		E	39.05	83.90	45.05	9.08*	11.79	65.43	90.78	1.62
1971	100	O	13	52	35		11	47	42	
		E	15.21	47.58	37.21	0.86	11.90	45.20	42.90	0.16
1980	100	O	16	50	34		5	39	56	
		E	16.81	48.38	34.81	0.11	6.00	37.00	57.00	0.29
1984-87	61	O	9	32	20		7	28	26	
		E	10.24	29.51	21.25	0.43	7.22	27.54	26.23	0.02

*P (df2) = 0.02-0.01.

tion in the zone studied. Moreover, the species composition has changed drastically with the introduction of invasive species like *D. subobscura*. The reasons why the observed changes were not followed by alterations in the genetic composition in *D. pavani* could be attributed to the characteristics of the chromosomal polymorphism in this species. One of the characteristics is the absence of marked geographic or seasonal variations of the different gene arrangement grequencies (Brncic, 1973). Thus in *D. pavani* the polymorphism seems to be "rigid", according to the definition given by Dobzhansky (1962) and commented on by Sperlich and Pfriem (1986). In contrast, *D. pseudoobscura* presents a more "flexible" polymorphism and geographical, seasonal and long-term changes in environment, are followed by changes in the frequencies of the different chromosomal orders. The rigid character of the chromosomal polymorphism in *D. pavani* is also inferred from the fact that in old laboratory stocks kept for more than 30 years, the gene arrangements still maintains frequencies near to those found in the natural populations (Brncic, 1969 and unpublished data). It was postulated that the stability of the inversion polymorphism in *D. pavani* is an

expression of the heterotic properties of the inversion heterozygotes. In fact, Table IV shows that in almost every sample the frequency of heterozygotes exceeds the expected values, estimated according to the Hardy-Weinberg distribution. The fact that the excess of heterozygotes are not statistically significant according to the Chi-square test, only reflects the lack of sensitivity of this method to detect heterozygote superiority or heterosis. However, the heterotic properties of the inversion heterozygotes in *D. pavani* have also been demonstrated under laboratory conditions for several traits associated with the fitness such as longevity, rate of development, mating activity and intra and interspecific competition (reviewed in Brncic, 1973). In short, the lack of response of the chromosomal polymorphism of *D. pavani* to drastic changes in the environment correlated with an abrupt reduction of the population size, could be interpreted as an additional expression of the heterotic properties of the inversion heterozygotes.

RESUMO

Durante os últimos 35 anos, mudanças drásticas nas populações de espécies nativas de *Drosophila pavani* foram detectadas em localidades suburbanas da região central do Chile. Antes de 1979, esta espécie era a mais abundante na área, mas atualmente é extremamente rara. Esta mudança pode ser atribuída a degradação do ambiente pelo crescimento da urbanização e coincide com a bem sucedida introdução de espécies como a paleartica *D. obscura*. Uma comparação da inversão de polimorfismo existente nas populações naturais de *D. pavani*, em amostras coletadas no mesmo local e estação em 55, 61, 71, 80 e 84-87, não mostrou diferenças na frequência dos arranjos distintos de genes. A extrema estabilidade do polimorfismo na espécie contrasta com aquela encontrada em outras espécies para as quais as mudanças no ambiente são seguidas por mudanças genéticas. A condição encontrada em *D. pavani* poderia ser interpretada como uma expressão das fortes propriedades heteróticas da inversão dos heterozigotos.

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