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URBANIZATION AND CHROMOSOMAL POLYMORPHISM OF *Drosophila nebulosa*

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ABSTRACT

The chromosomal polymorphism of one wild and eight urban populations of *Drosophila nebulosa* from Porto Alegre, in Southern Brazil, were studied in order to verify the relationship with the degree of urbanization of the sampling sites. The results suggest that the polymorphism estimates are not related to the urbanization zones defined for the city. Probable correlations with microenvironmental characteristics of the sampling sites are discussed.

INTRODUCTION

Studies on *Drosophila* species have largely documented the adaptive role of chromosomal polymorphism as well as its regulation by selective, non-alleatory factors. Today, with the striking and continuous alterations imposed by men to the environmental, the evaluation of the genetic consequences of this process is a subject of paramount importance. For this study, paracentric inversions in polytene chromosomes stand as privileged material.

In spite of the large number of insect species that colonize and breed in man-made environments, there has not been much systematic work on the adaptation genetics of these organisms to such altered habitats. Dubinin and Tiniakov's (1945, 1946a,b, 1947) and Borisov's (1969) studies on the cosmopolitan species *Drosophila funebris* in urban and suburban areas of Moscow and neighboring country sites found an

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increase in the number of heterozygotes for inversions from the periphery toward the center of the city. It seemed evident that urban environments offered favorable conditions for the spread of inversions, while in rural areas the standard arrangements predominated. The opposite tendency was found for the colonizing wild species, *D. willistoni* in Southern Brazil, by Valente *et al.* (1989). A decrease of chromosomal polymorphism was detected from the periphery toward the center of the city.

We studied another species of the *willistoni* group, the non-sibling *D. nebulosa*. Contrary to the other species of this group, which demonstrate a clear affinity for humid climates and forest zones, *D. nebulosa* is dominant in regions with a prolonged dry season, such as savanas and caatingas; however, in regions with a more balanced distribution of rainfall, it rarely becomes very abundant (Dobzhansky and Pavan, 1950).

This species probably has a neotropical origin, and is distributed from Southern South America to Texas and Nebraska, USA (Ehrman and Powell, 1982). Its center of origin, however, is not known. As this so-called wild species has been found, although in low frequencies, in samples of rotting fruits fallen from trees in several points of Porto Alegre city, Southern Brazil (Bonorino and Valente, 1989) we decided to study the chromosomal polymorphism in urban populations of this fly. We sought for differences that could be related to the urbanization levels of the sampling environments.

MATERIAL AND METHODS

Studies were made in the city area of Porto Alegre (1.300.000 inhabitants), the capital of the state of Rio Grande do Sul, in Southern Brazil (51°06'W - 30°10'S). The region has a wet subtropical climate with high temperatures in the summer. The annual mean temperature is 19.5°C, and the annual rainfall is 1,317 mm. The city shows a radial urbanization gradient, determined by the radial distribution of the main avenues. This causes a complementary gradient of vegetation (Ruszczyck, 1986; 1986/1987).

Five transect routes were traced along the principal avenues (Figure 1) from the center toward the periphery, crossing the three main urbanization zones previously identified in our city, according to the method of Ruszczyck (1986). This method can be summarized as follows: "by examination of 1:20,000 aerial photographs of the city with the aid of a stereoscope, three zones of different intensities of urbanization could be drawn over a political map at the same scale: high (building zone), with vegetation cover below 20%, zero to 2 km distant from the center of the city; medium (house and building zone), vegetation cover between 20 and 40%, 2 to 7 km distant from the center of the city; and low (house zone), vegetation cover above 40%, 4 to 12 km distant from the center of the city. The borders of the zones were adjusted by examination *in loco*. The final map was simplified to poligons, by drawing tangential lines to the borders of the different zones of urbanization." (Ruszczyck, 1986).

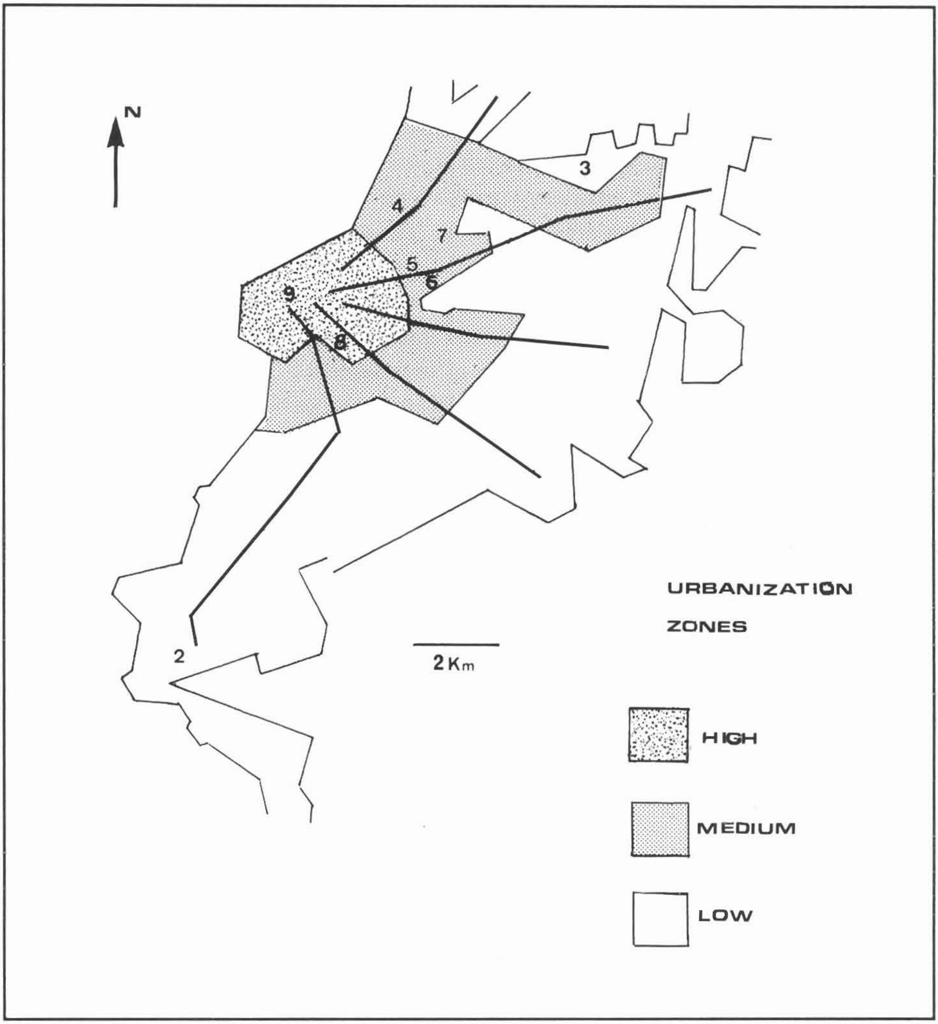

Figure 1 - Simplified map of the city of Porto Alegre. The three main urbanization zones as identified by Ruszczyck (1986) are indicated. Lines represent the five main transect routes of collection. Numbers indicate the locations of the sampling sites: 1, Wild environment (outside of the figure); 2, Mario Totta St.; 3, Deoclecio Pereira St.; 4, Pinheiro Machado Sq.; 5, Bordini St.; 6, Goethe Av.; 7, Mauricio Cardoso Sq.; 8, Piratini Sq; 9, Farroupilha Park.

Of the eight urban samples analysed, two came from the high urbanization zone, four from the medium and two from the low urbanization zone (Figure 1). A ninth sample, from a wild environment (the Agronomic Experimental Station of Guaiba, 51°30'W -

30°05'S, 40 km from Porto Alegre) with a vegetation that resembles the pre-urban environment of the city, was included for analysis, in order to compare the chromosomal structure of that wild population with the urban ones.

At the sampling sites, rotting fruits fallen from trees and bushes were collected. This material, supposed to contain preadult forms of *Drosophila* was brought to the laboratory in plastic bags and distributed in vials with standard culture medium (Marques *et al.*, 1966) with baker's yeast diluted in water. The emergent adults were collected by an aspirator, identified and counted.

The females of *D. nebulosa* were distributed as isofemale strains, in individual vials containing the same culture medium and diluted yeast. The F1 third instar female larvae were processed by the cytological technique of Ashburner (1967). The chromosomal arrangements detected were compared to those described by Pavan (1946) and to photomicrographs described by Regner *et al.* (1991).

The Kruskal-Wallis test, followed by multiple comparisons (Daniel, 1978) was employed to compare different collection sites for the mean number of inversions. For the percentage of heterozygotes, a chi-square test was performed. All the available data (frequencies of eight detected chromosomal arrangements, percentage of heterozygotes and mean number of inversions) were employed to evaluate the similarities among samples of the different collection sites, through a cluster analysis. The Manhattan distance was used as the dissimilarity coefficient for the UPGMA (unweighted pair-group method using arithmetic averages) clustering algorithm (Sneath and Sokal, 1973).

RESULTS

Table I describes the samples and presents the estimates of chromosomal polymorphism of the populations analysed. The small sample sizes are exclusively due to the reduced dimensions of the local population of *Drosophila nebulosa* (Bonorino and Valente, 1989), and did not depend on our sampling efforts.

In more than 285 samples obtained in Porto Alegre after 1987, we have never obtained more than 15 adult individuals of *D. nebulosa* per sample. Among the city samples, the mean number of inversions per female ranged from 0.40 to 1.37. The percentage of heterozygotes varied between 25.0 and 75.0. The wild environment samples presented intermediary values (0.90 and 60.0, respectively).

The eight chromosomal arrangements that accounted for the observed polymorphisms and their frequencies are indicated in Table II. The frequencies varied considerably among the sampling sites, inversions B and H showing the highest values (mean: 31.4 and 30.6, respectively).

The variation in the degree of heterozygosis for inversions among the samples was evaluated by means of a chi-square test and a Kruskal-Wallis test (mean number of

Table I - Estimates of the degree of chromosomal polymorphism of one wild and eight urban populations of *Drosophila nebulosa*.

Sampling sites	Urbanization level	Collection date	Number of analysed individuals	Heterozygotes (%)	Mean no. of inversions per female ± standard deviation
Wild Environment	wild	04/87	50	60.0	0.90 ± 0.90
Mario Totta St.	low	04/87	43	74.4	1.35 ± 1.09
Deoclecio Pereira St.	low	03/85	24	58.3	1.37 ± 1.41
Pinheiro Machado Sq.	medium	03/87	35	65.7	0.94 ± 0.87
Bordini St.	medium	03/87	10	60.0	0.80 ± 0.79
Goethe Av.	medium	05/87	68	38.2	0.53 ± 0.73
Mauricio Cardoso Sq.	medium	06/85	24	75.0	1.33 ± 1.09
Piratini Sq.	high	04/87	20	25.0	0.40 ± 0.75
Farroupilha Park	high	04/87	50	60.0	0.94 ± 0.98
All samples	-	-	324	$\bar{x}$ - 56.5	$\bar{x}$ - 0.95 ± 0.35

inversions per female). The chi-square value (x^2 was 28.087, with 9 degrees of freedom (df) ($P < 0.001$) indicated significant differences among the populations. It was probably due to the observed low percentages of the Piratini Sq. (25%) and Goethe Av. (38.2%)

Table II - Frequencies of heterozygotes for chromosomal arrangements detected in one wild and eight urban populations of *Drosophila nebulosa*.

Sampling sites	Frequencies of gene arrangements heterozygotes								(%) No. of individuals analysed
	A	B	C	G	H	I	L	G+H	
Wild Environment	04	38	04	06	36	00	02	00	50
Mario Totta St.	09	46	14	12	39	00	09	00	43
Deoclecio Pereira St.	29	25	29	04	21	00	08	17	24
Pinheiro Machado Sq.	06	57	00	29	46	00	06	11	35
Bordini St.	00	10	00	30	30	00	10	00	10
Goethe Av.	00	12	08	25	42	00	03	00	68
Mauricio Cardoso Sq.	12	46	17	12	12	04	04	17	24
Piratini Sq.	00	15	00	25	00	00	00	00	20
Farroupilha Park	02	34	10	12	24	00	02	00	50
Total	6.9	31.4	9.11	14.4	30.6	-	4.9	6.1	324

samples, on one hand, and the high figures obtained for the Mario Totta St. (74.4%) and the Mauricio Cardoso Sq. (75%) samples, on the other.

Table III presents the results of the Kruskal-Wallis tests performed on the mean number of inversions per female for samples of the same urbanization zone. While the two samples from the low urbanization zone did not differ from each other, the two from the high urbanization zone differ significantly (x^2 = 5.554, df = 1; P < 0.05). This is not surprising, for while the Mario Totta and the Deoclecio Pereira samples come from relatively similar environments (respectively, an orchard and the backyard of a house), the Farroupilha Park, although located in the center of the city and inside the high urbanization zone, is a park of 170,000 m^2, and constitutes the largest vegetation area inside the city. Danni (1980) has shown that the temperature inside the park is cooler (and humidity levels are higher) inside the park than outside it, in the neighborhoods. On the other hand, the Piratini Sq. is a small square, has very few trees and bushes, and is surrounded by heavy traffic.

Table III - Results of the Kruskal-Wallis test and nonparametric multiple comparisons among samples of the same urbanization zone, considering the average number of inversions per female.

Urbanization zone	No. of samples	No. of individuals analysed	K-W x^2	P	Nonparametric multiple comparisons
Low	2	67	0.024	> 0.80	-
Medium	4	137	14.756	< 0.01	G B P M * ---- ----------
High	2	70	5.554	< 0.05	-

* G - Goethe Av.; B - Bordini St.; P - Pinheiro Machado Sq.; M - Mauricio Cardoso Sq.; underlined samples do not differ significantly from each other.

Inside the medium urbanization zone, the samples also differed significantly. Nonparametric multiple comparisons showed that the Goethe Av. sample departed from the Pinheiro Machado and Mauricio Cardoso ones. The Bordini population presented intermediary values, not differing from the others.

Table IV summarized the results of the Kruskal-Wallis test performed among all samples, but considering the results shown in Table III (Goethe separated from the medium urbanization zone; Farroupilha and Piratini considered apart). As expected, the populations differed significantly (x^2 = 28.864; df = 6; P < 0.001) for the mean number of inversions. Nonparametric multiple comparisons revealed that there is no significant difference between the samples from the low and medium urbanization zones, as well as between the Goethe Av. and the Piratini samples. The populations from the wild environment and Farroupilha Park showed intermediary positions.

Table IV - Mean number of inversion heterozygotes per female: results of the Kruskal-Wallis test and nonparametric multiple comparisons considering the Goethe Av. sample apart from the medium urbanization zone and separating the two samples of the high urbanization zone (see Table III). The wild environment sample is also included.

	Samples					
	Piratini	Goethe	wild	Farroupilha	Medium urbanization zone	Low urbanization zone
K-W Mean Rank	112	127	163	165	178	195*
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Mean no. of inversions	0.40	0.53	0.90	0.94	1.06	1.36
No. of individuals	20	68	50	50	69	67

x^2 - 28.864; P < 0.001

* Underlines indicate samples that do not differ significantly from each other.

Since no association was found between the average estimates of chromosomal polymorphism and the urbanization zones, we performed a cluster analysis among the samples, in an attempt to evaluate the similarities among them. All the available polymorphism data (mean number of inversions, percentage of heterozygotes, and frequencies of the chromosomal arrangements) were employed. The results are presented in Figure 2. Clearly, the samples do not cluster according to the urbanization zone they belong to. However, when we consider other characteristics of the surrounding environment of the sampling sites, an interesting pattern seems to emerge.

The first two samples to group come from the wild environment (1) and the Farroupilha Park (9), (Manhattan distance (MD) = 4.12), which are large vegetation areas. The next cluster groups samples 5 (Bordini St.) and 6 (Goethe Av.) (MD = 6.58), both coming from palm trees situated on streets with heavy traffic. This group subsequently clusters with Piratini Sq. (8), where the environment is rather similar, as discussed above. At a distance of approximately 9.50, three clusters were identified. The first groups sampling sites with surrounding vegetation areas: wild environment, Farroupilha Park, Mario Totta St. (an orchard), Mauricio Cardoso Sq. and Pinheiro Machado Sq. (squares with many trees, in streets bearing little traffic). The second clusters the samples from

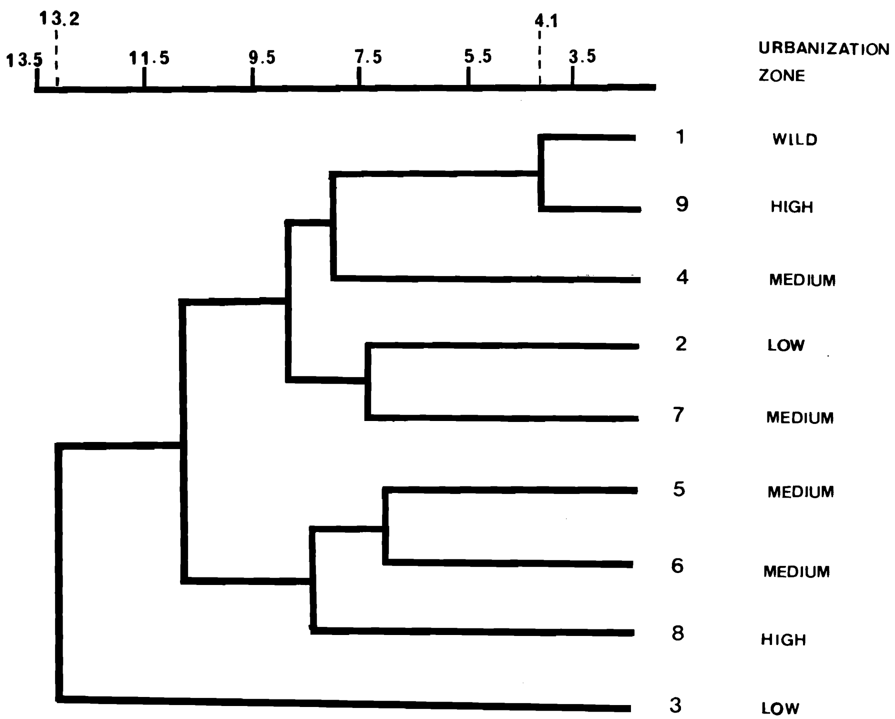

Figure 2 - Dendrogram resulting from the UPGMA analysis performed with all samples, employing all the polymorphism data (mean number of inversions per female, percentage of heterozygotes, and frequencies of the chromosomal arrangements). Numbers represent the sampling sites: 1, Wild environment; 2, Mario Totta St.; 3, Deoclecio Pereira St., 4, Pinheiro Machado Sq.; 5, Bordini St.; 6, Goethe Av.; 7, Mauricio Cardoso Sq.; 8, Piratini Sq.; and 9, Farroupilha Park.

isolated trees, surrounded by traffic. Finally, the third cluster is formed by a single sample, the one from Deoclecio Pereira St., which comes from another different environment: the backyard of a house.

DISCUSSION

If chromosomal polymorphism is adaptive, a species should be more polymorphic the nearest it is to the center of origin, as well as to available resources (as discussed for instance in Da Cunha *et al.*, 1950). Our urban populations of *D. nebulosa*, although rather far from the places where this species attains its highest frequencies (xeric savanas and caatingas of Central Brazil) show average numbers of inversions comparable to those of populations from such environments (Da Cunha *et al.*, 1953). Interestingly, cities have already been called "urban savanas" (Brady *et al.*, 1979).

Studies comparing the chromosomal polymorphism of species from urban and non-urban environments (Dubinin and Tiniakov, 1945, 1946a,b, 1947; Borisov, 1969; Valente *et al.*, 1989; Singh and Das, 1990) have detected differences that could be related to the urbanization of the environment. Just how precisely could this tendency be evaluated?

When we tried to relate the chromosomal polymorphism estimates of *D. nebulosa* to the main urbanization zones defined for our city, no association was found. However, when we analysed the results of the UPGMA cluster analysis we observed that populations sites with a greater vegetation cover and little disturbance by traffic appeared to be more similar, while the ones from places surrounded by streets and traffic grouped together. The sample from the backyward of a house in a residential zone showed no similarities to either of the other two groups.

Although the action of stochastic factors on small populations cannot be ruled out, our results seem to suggest that the microenvironment may play a major role on the adaptation of this fly to urban areas. Such microenvironments, with their particular microclimates, resource availability, differential presence or absence of competitors, etc, could be diluted in the urbanization criterium used by us (Ruszczyck, 1986; 1986/1987), which is heavily dependent on the percentage of constructed area (area occupied by buildings and/or houses). Different urbanization zones could certainly contain similar microenvironments.

The possibility that the environmental modifications due to traffic could affect chromosomal polymorphism is also interesting. Traffic is known to influence the levels of polution and humidity of the air (e.g. Brady *et al.*, 1979). Besides suffering these indirect influences through environmental changes, a population surrounded by traffic could also face limitations to its expansion. The non-availability of new resources to colonize, and even the difficulty to migrate, could lead to modifying influences such as inbreeding and drift.

As a consequence of our present findings, studies are currently being made in an attempt to identify microenvironmental characteristics of each sample site as well as to evaluate levels of disturbances caused by traffic to these different places of Porto Alegre city. These studies aim to improve our criteria for screening environmental differences among the sampling sites.

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RESUMO

Foi estudado o polimorfismo cromossômico de populações de *Drosophila nebulosa*, das quais, uma procedente de um local selvagem e oito de sítios urbanos da cidade de Porto Alegre, no sul do Brasil, com o objetivo de verificar uma possível relação entre esse marcador e o grau de urbanização dos sítios de coleta.

Os resultados sugerem que as estimativas de polimorfismo não estão relacionadas com as zonas de urbanização da cidade. Uma possível correlação entre o polimorfismo cromossômico e as características de microambientes dos sítios de coleta é discutida.

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