

Cytological maps and chromosomal polymorphism of *Drosophila polymorpha* and *Drosophila cardinoides*

Cláudia Rohde and Vera Lúcia S. Valente

ABSTRACT

Four urban populations of the wild species *Drosophila cardinoides* and one of *Drosophila polymorpha* from Porto Alegre city, and one of each species from a natural control location (Eldorado do Sul), in Southern Brazil, were analyzed for the presence of paracentric inversions in their polytene chromosomes. Reference maps of the salivary gland chromosomes of both species were constructed and chromosomal polymorphism was recorded and qualitatively analyzed. In *D. polymorpha* populations, only one inversion was detected on chromosome arm IIR, whereas in *D. cardinoides*, despite the small size of the samples studied, four heterozygous inversions were identified on chromosome arm IIII, plus the homozygous arrangement. Certain evolutionary implications of these findings are discussed.

INTRODUCTION

Drosophila polymorpha and *Drosophila cardinoides*, both described by Dobzhansky and Pavan (1943), belong to the *Drosophila cardini* species group. They are closely related and morphologically very similar, except for the light thorax pigmentation of *D. polymorpha*. The two species live in sympatry and are ubiquitous in Neotropical communities from Northern Argentina to Central America (Val *et al.*, 1981), and have been studied from ecological, enzymatic and evolutionary points of view (Da Cunha *et al.*, 1953; Napp and Cordeiro, 1978, 1981; Rohde, 1990).

Cytologically, they are also very similar, since their metaphase configurations (Dobzhansky and Pavan, 1943) consist of two metacentric pairs (chromosomes II and III), one punctual pair (IV) and an acrocentric sex pair, with the Y being heterochromatic.

Although previously studied by Da Cunha *et al.* (1953) and by Heed and Russell (1971), their inversion chromosomal polymorphism was not recorded on plates and the break points of the inversions found were not indicated, thus preventing more accurate studies about these species.

MATERIAL AND METHODS

Flies were sampled using conventional banana baits with entomological nets or by collecting several rotting fruits colonized by preadult forms (eggs, larvae and pupae) of *Drosophila*, inside Porto Alegre city squares and gardens (30°10'S; 50°05'W), and in a natural location, Eldorado do Sul (30°05'S; 51°39'W), roughly 40 km from the city, during 1990.

Metaphase plate chromosomes of brain ganglia of early 3rd instar larvae of *D. polymorpha* and *D. cardinoides* were processed by the technique of Guimarães and Bicudo (1982). Photomaps of the

polytene chromosomes of *D. polymorpha* and *D. cardinoides* were constructed after the analysis of several F1 third instar larvae of each isofemale strain, processed cytologically according to the technique of Ashburner (1967). The inversion polymorphism detected in heterozygosity in the chromosomes of both species, from one F1 larva of each isofemale strain, was only analyzed qualitatively due to the small number of specimens captured in each of the four urban samples of *D. cardinoides* and one of *D. polymorpha*, and at the control location (one sample for each species). Despite the

ubiquity of both species in urban environments the number of individuals captured was always small, despite considerable effort to obtain larger samples.

RESULTS AND DISCUSSION

Figures 1 and 2 correspond to the photomaps of *D. polymorpha* and *D. cardinoides*, respectively, containing the break points of the heterozygous inversions found in the populations sampled. The cell

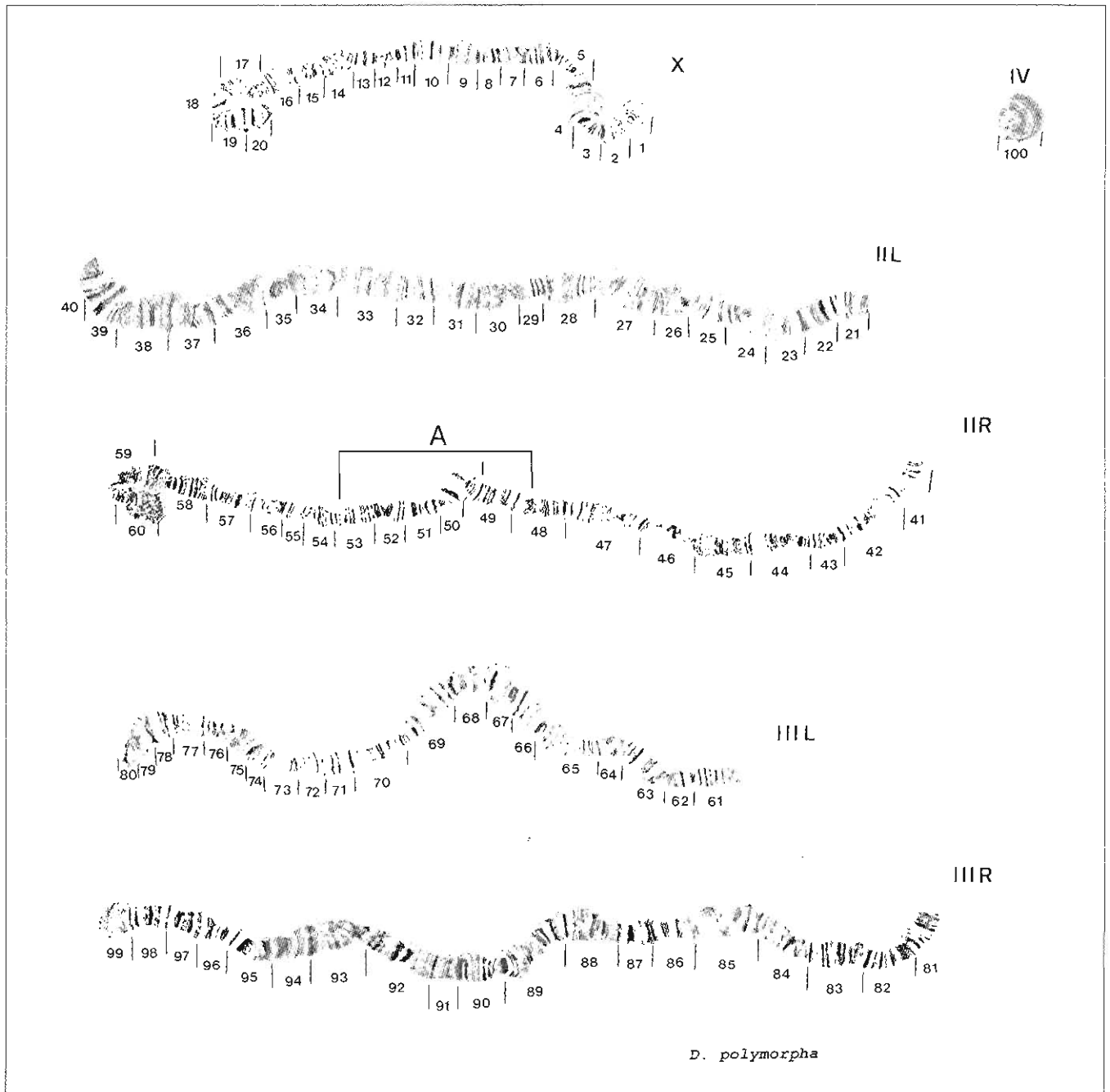


Figure 1 - Chromosome map of *Drosophila polymorpha* with the inversion A break points. The tips of all chromosome arms are reported at the right side of the figure and the letters L and R correspond, respectively, to the left and right arms of the same chromosome.

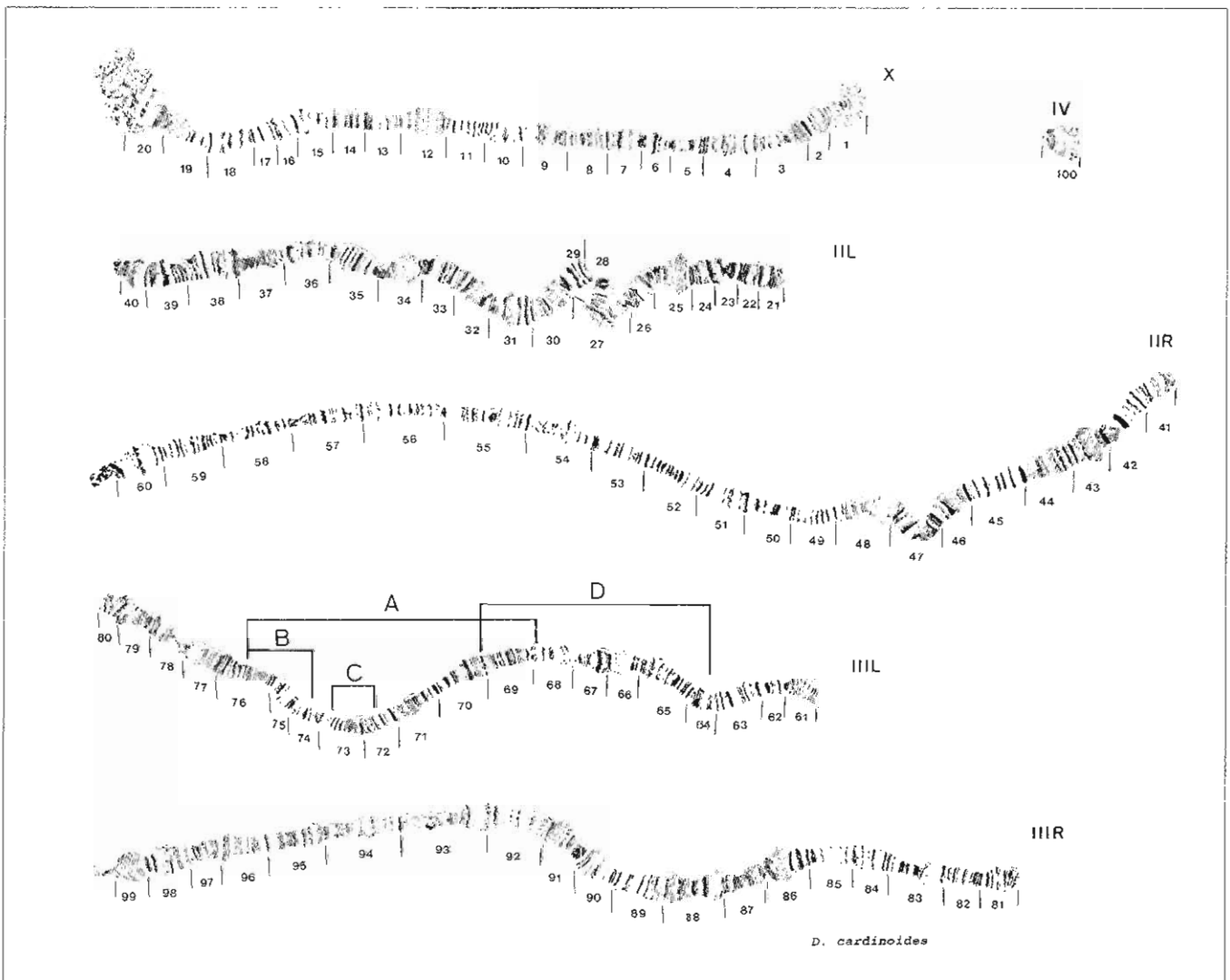


Figure 2 - Chromosome map of *Drosophila cardinoides* with the inversions A, B, C and D break points. The tips of all chromosome arms are reported at the right side of the figure and the letters L and R correspond, respectively, to the left and right arms of the same chromosome.

nuclei of the salivary glands of both species exhibit five long arms, plus one punctual, very short chromosome. The establishment of correspondence between salivary gland chromosomes and brain ganglion metaphase plates (Figure 3) was possible due to the differences found in size and the centromere position among the pairs.

The photomaps (Figures 1 and 2) clearly show the similarity of the banding patterns of the two species, although in certain regions, especially on the IIR chromosome arm, the homology was lost. Probably structural rearrangements occurred in each species during their evolutionary history and regulatory differences visible at the puffing level may be responsible for such apparent dissimilarities.

The polytene chromosomes of *D. polymorpha* (and also those of *D. cardinoides*) were divided arbitrarily into 100 sections of similar size, as usual

starting at the tip of the X chromosome (section 1) and ending at the base of chromosome IV (section 100). Chromosomes II and III were divided into left and right arms as IIL, IIR, IIIL and IIIR, respectively. The same was done for the corresponding chromosomes of *D. cardinoides* (Figure 2).

Chromosome X was the smallest one in both species, showing large puffs in sections 4 and 16 of *D. polymorpha*. The tip of the X chromosome is puffed in the two species and contains a large proportion of the heterochromatin. It was divided into 20 sections and was recognized by its light appearance in male larvae.

Chromosome II, although observed as metacentric in metaphase brain plates of both species, appeared to have arms of similar size only in *D. polymorpha*. In *D. cardinoides* the second chromosome seemed to have arms of different size, with the IIR being the longest at the polytene level. Nevertheless, they

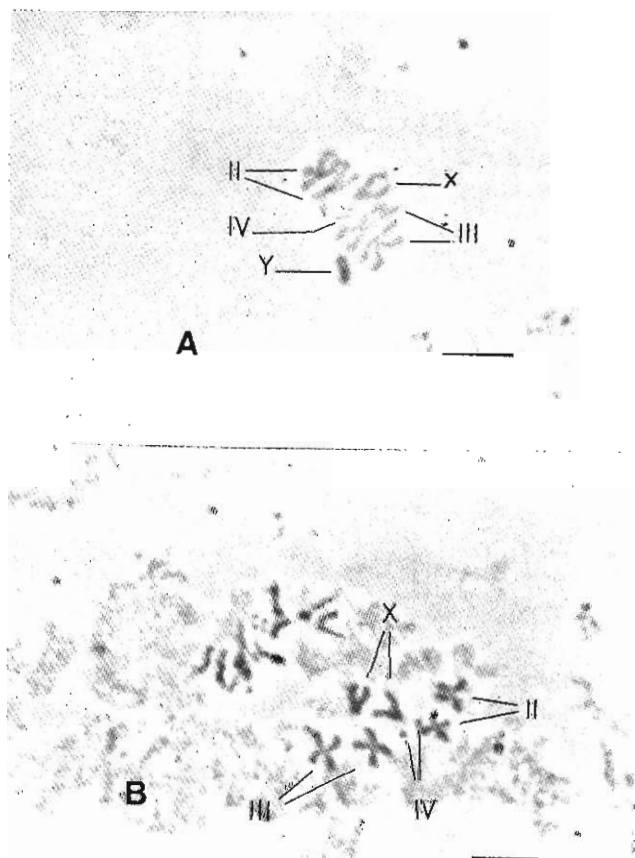


Figure 3 - Metaphase plates of the brain ganglion of a *Drosophila polymorpha* male (A) and a *D. cardinoides* female (B). Bar represents 10 µm.

were both divided into the same number of sections (20), with 21 being located at the tip of IIL, 40 at its base, 41 at the tip of IIR, and 60 near the chromocenter.

Diagnostic constrictions were observed especially in sections 35, 30 and 26 in *D. polymorpha* chromosomes, and in sections 37, 35, 28 and 23 in *D. cardinoides*. Large puffs were also observed in sections 34 and 35 of *D. cardinoides*.

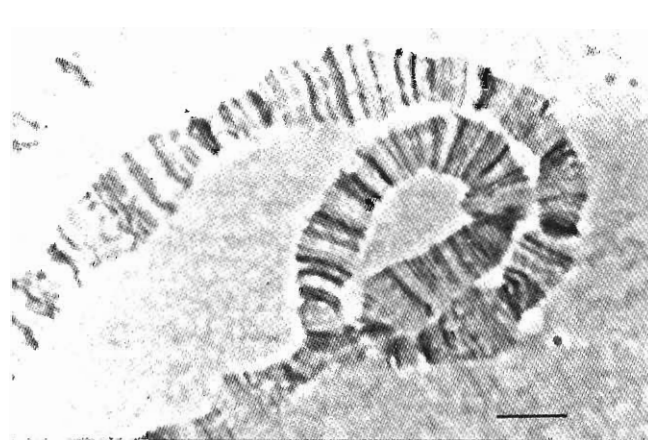


Figure 4 - IIR-A inversion of *Drosophila polymorpha*. Bar represents 10 µm.

As was the case for the second chromosome, chromosome III, in most preparations, seemed to have arms of different size, despite the small difference detected between brain ganglion chromosomes of *D. polymorpha* and *D. cardinoides*. Thus, we propose that pairs II and III should be considered as submetacentric both in *D. polymorpha* and *D. cardinoides*. Chromosome III covered sections 61 to 80 from the tip to the base of the left arm (IIIL) and 81 to 99 of the right arm (IIIR) in both two species. Hallmarks identified in these chromosomes were the puffed sections 68 and 67 of *D. polymorpha*, apparently corresponding to 67 and 66 of *D. cardinoides*, and the bulb shape of the base (section 99), separated from section 98 by two well-defined bands.

Finally, the small chromosome IV was composed of a single section (100), more flattened in *D. cardinoides* and rounded in *D. polymorpha*.

The photomicrographs of *D. polymorpha* and *D. cardinoides* inversions are presented in Figures 4 and 5, respectively. Table I shows the distribution of chromosomal variants by collection site, classified by

Table I - Inversions present in samples of *Drosophila polymorpha* and *Drosophila cardinoides* from Porto Alegre city and from a control location.

| Location | Urbanization level | Inversions | | | |
|-----------------------|--------------------|----------------------|----|------------------------------|----|
| | | <i>D. polymorpha</i> | N | <i>D. cardinoides</i> | N |
| Porto Calvo St. | Low | IIR-A, HOM. | 06 | IIIL-A, IIIL-D, HOM. | 43 |
| CEASA | Low | - | - | IIIL-A, HOM. | 10 |
| Deoclecio Pereira St. | Low | - | - | IIIL-B, IIIL-C, IIIL-D, HOM. | 06 |
| Mauricio Cardoso Sq. | Medium | - | - | HOM. | 12 |
| Eldorado do Sul | Control* | IIR-A, HOM. | 07 | IIIL-A, HOM. | 16 |

N = Number of isofemales analyzed.

HOM. = Homozygous standard chromosome form.

Urbanization level according to Rusczyk (1986/1987). Control* corresponds to a natural woodland outside the urban area of Porto Alegre.

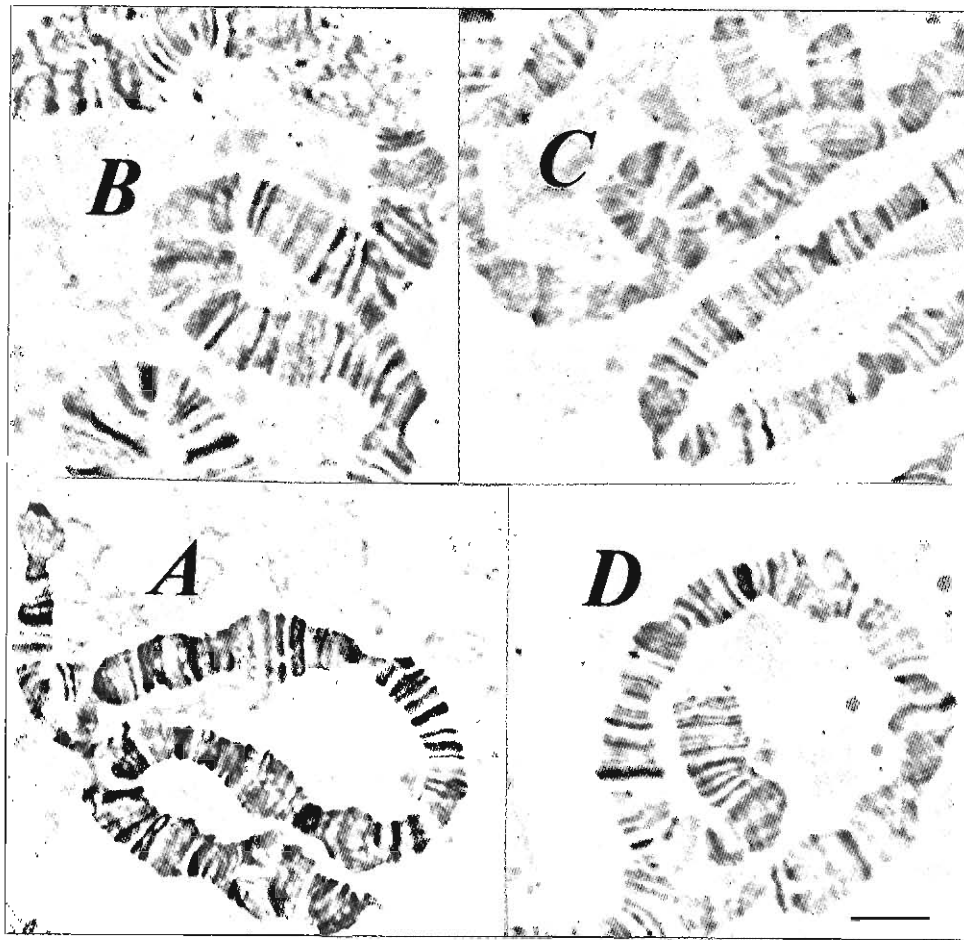


Figure 5 - Chromosome III of *Drosophila cardinoides* with inversions A, B, C and D. Bar represents 10 μ m.

urbanization level, according to the criterion of Rusczyk (1986/1987) - based mainly on the proportions of green and constructed areas obtained from 1:20,000 aerial photographs and *in loco* observations.

In spite of the small number of larvae analyzed, which reflects the low density of the populations, it can be seen that *D. cardinoides* was highly polymorphic in our samples (only the Mauricio Cardoso Square sample of this species was monomorphic). Among the few specimens analyzed of *D. polymorpha*, only one variant (IIR-A inversion) was observed in two samples, one from Porto Calvo Street, located at a more peripheral point of the city, and the other from the control location.

If we consider the urbanization level of the sampling sites, the inversion IIII-A of *D. cardinoides* appears to be confined to more peripheral sites, whereas the sample from the most urbanized place (Deoclecio Pereira Street, which consists of a residential garden) harbored three variants, plus the homozygous chromosome forms. Due to the small sample size, the possibility of association between disturbed areas and certain inversions could not be determined.

D. polymorpha is apparently a better colonizer species of the urban environment (Rohde, 1990; Rohde and Valente, in press) than *D. cardinoides*. The association between high colonizer propensity and low chromosomal polymorphism is theoretically supported by the classic works of Da Cunha and Dobzhansky (1954), Da Cunha *et al.* (1959), and Carson (1955, 1965) that suggest that the degree of polymorphism of certain species is a function of the number of niches exploited. The lack of chromosomal variability along the geographic borders of the species distribution seems to be the result of selective factors favoring structural homozygosis and the consequent increase of recombination (Carson, 1955, 1959; Da Cunha *et al.*, 1959).

RESUMO

Quatro populações urbanas da espécie selvagem *Drosophila cardinoides* e uma da espécie *Drosophila polymorpha*, encontradas na cidade de Porto Alegre e em um local controle

(Eldorado do Sul) no sul do Brasil, foram estudadas quanto à organização de seu cariótipo e quanto à presença de inversões paracêntricas em seus cromossomos politênicos. Para tanto, foram confeccionados fotomapas de referência dos cromossomos presentes nas células da glândula salivar de ambas as espécies e o polimorfismo cromossômico foi analisado qualitativamente em todas as amostras. *D. polymorpha* apresentou uma inversão em heterozigose no cromossomo IIR, além do arranjo homocigoto, e *D. cardinoides*, apesar do pequeno tamanho das amostras, apresentou quatro inversões no cromossomo IIIL, além do arranjo homocigoto. Algumas implicações evolutivas desses achados são discutidas.

REFERENCES

- Ashburner, M. (1967). Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. I. Autosomal puffing patterns in a laboratory stock of *Drosophila melanogaster*. *Chromosoma* 27: 47-63.
- Carson, H.L. (1955). The genetics characteristics of marginal populations of *Drosophila*. *Cold Spring Harbor Symp. Quant. Biol.* 20: 276-287.
- Carson, H.L. (1959). Genetic conditions which promote or retard the formation of species. *Cold Spring Harbor. Symp. Quant. Biol.* 24: 87-105.
- Carson, H.L. (1965). Chromosomal morphism in geographically widespread species of *Drosophila*. In: *The Genetics of Colonizing Species*. (Baker, H.G. and Stebbins, G.L., eds.). Academic Press, New York, pp. 508-531.
- Da Cunha, A.B. and Dobzhansky, T. (1954). A further study of chromosomal polymorphism in *Drosophila willistoni* in its relation to environment. *Evolution* 8: 119-134.
- Da Cunha, A.B., Brncic, D. and Salzano, F.M. (1953). A comparative study of chromosomal polymorphism in certain South American species of *Drosophila*. *Heredity* 7: 193-202.
- Da Cunha, A.B., Dobzhansky, T., Pavlovsky, O. and Spassky, B. (1959). Genetics of natural populations. XXXVIII. Supplementary data on the chromosomal polymorphism in its relations to the environment. *Evolution* 13: 389-404.
- Dobzhansky, T. and Pavan, C. (1943). Studies on Brazilian species of *Drosophila*. *Biologia Geral* 4. *Bol. Fac. Cienc. e Letr. Univ. S. Paulo (Brazil)* 36: 7-72. *Biologia Geral* 4.
- Guimarães, M.C. and Bicudo, H.E.M.C. (1982). Yeast also increases the frequency of metaphases in *Drosophila* brain. *Braz. J. Genet.* 2: 433-436.
- Heed, W.B. and Russell, J.S. (1971). Phylogeny and population structure in island and continental species of the *cardini* group of *Drosophila*. Studies by inversion analysis. *Univ. Texas Publ.* 7103: 91-130.
- Napp, M. and Cordeiro, A.R. (1978). Heterosis in a wild strain of *Drosophila polymorpha* with a lethal closely linked to the major esterase locus. *Bioch. Genet.* 16: 609-617.
- Napp, M. and Cordeiro, A.R. (1981). Interspecific relationship in the *cardini* group of *Drosophila* studied by electrophoresis. *Braz. J. Genet.* 4: 537-547.
- Rohde, C. (1990). Polimorfismo cromossômico e variações ambientais em populações naturais de *Drosophila polymorpha* Dobzhansky and Pavan e de *Drosophila cardinoides* Dobzhansky and Pavan na cidade de Porto Alegre (RS). Master's thesis. Universidade Federal do Rio Grande do Sul, Porto Alegre, RS.
- Rohde, C. and Valente, V.L.S. Ecological characteristics of urban populations of *Drosophila polymorpha* Dobzhansky & Pavan and *Drosophila cardinoides* Dobzhansky & Pavan (Diptera, Drosophilidae). *Braz. J. Entom.* (in press).
- Ruszczyk, A. (1986/1987). Distribution and abundance of butterflies in the urbanization zones of Porto Alegre, Brazil. *J. Res. of Lepidop.* 25: 157-178.
- Val, F.C., Vilela, C.R. and Marques, M.D. (1981). Drosophilidae of the Neotropical region. In: *The Genetics and Biology of Drosophila* (Ashburner, M., Carson, H.L. and Thompson Jr., J.N., eds.). Vol. 3A. Academic Press, London, pp. 123-168.

(Received October 20, 1994)