Stegana Meigen

Zaprionus Coquillett

coracina Kikkawa and Peng, 1938
hirsuata Singh and Dash, 1999 (in press)
nainitalensis sp. nov. (this paper)
indianus Gupta, 1970 (for Indiana)

Further evidence of long-term persistence of inversion polymorphism in laboratory strains of *Drosophila melanogaster*.

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Abstract: In the present study seven laboratory stocks of *Drosophila melanogaster* raised from females collected from different localities in Brazil, between 1995-1996, were analyzed with the objective to evaluate the frequency of heterokaryotypes maintained under laboratory conditions. After at least two years of maintenance in laboratory, under standard conditions at 25°C, the frequency of heterokaryotypes were maintained at moderate (23.9 to 31.6%) to significantly high (56.7 and 58.1) frequencies.

Introduction

Quantitative variation in the frequency of common cosmopolitan inversions in geographic populations of *D. melanogaster* from various regions of the world has been reported by different investigators. The superior fitness of heterokaryotypes is one type of balancing selection (Wright and Dobzhansky, 1946; Dobzhansky, 1947) used to explain the maintenance of inversion polymorphism in natural populations of *Drosophila*. Frequency-dependent selection (Kojima and Tobari, 1969; Nassar et al., 1973; Anderson and Watanabe, 1974; Gromko and Richmond, 1978; Anderson et al., 1986) and supergene selection (Wasserman, 1968, 1973, 1975) are others.

Polymorphism in *D. melanogaster* has been characterized as "flexible". As fitness of homo and heterokaryotypes depends on the environment conditions in species with "flexible" polymorphism, *D. melanogaster* tends to lose inversions and to become monomorphic for the standard gene arrangement in laboratory conditions. Inoue (1979) reported that polymorphic inversions of *D. melanogaster* found in high frequency in nature were almost eliminated after about twenty months in laboratory populations maintained in population cages, suggesting that inversions are disadvantageous compared to standard chromosomes under these conditions.

In the present study seven stocks of *D. melanogaster* raised from females collected from different localities in Brazil between 1995-1996 were analyzed with the objective to evaluate the frequency of heterokaryotypes maintained under laboratory conditions.

Material and Methods

The flies captured during 1995 and 1996 were used to set up seven strains are: MA, from São Luís (MA, 2°31'S Lat), set up with seven females; PI, from Teresina (PI, 5° 09'Slat); SP, from São José do Rio Preto (SP, 20°49'S Lat); PR, from Maringá (PR, 23°25'S Lat); SC, from Joinville (SC,

26°18'S Lat) and RS, from Porto Alegre (RS, 30°02'S Lat). The strains were kept in bottles containing banana-agar culture medium at room temperature, at 25°C ± 1°C. Between 1998 and 1999, smears of salivary gland chromosomes were prepared with F1 third instar larvae from individual crosses of four day old virgin couples. The chromosome preparations were made by lactic-acid-orcein method and then observed under microscope for the presence of heterokaryotypes.

Results

The occurrence of heterokaryotypes in larvae of 26 up to 43 couples was scored (Table 1, Figure 1). It was detected the presence of five types of heterokaryotypes. They were constituted by the inversions *In(2L)22D;34A* (Figure 1c), *In(2R)52A;56F* (Figure 1b), *In(3L)63C;72F* (Figure 1d), *In(3L)66C;73A* (Figure 1e), *In(3R)89D;96A* (Figure 1f). There were not found inversions in chromosome X (Figure 1a).

Table 1. Frequencies of heterokaryotypes and homokaryotypes for five cosmopolitan inversions, and total, in seven laboratory strains of *D. melanogaster* proceeding from different geographic regions of Brazil.

Inversion	MA		PI		MG		SP		PR		SC		RS	
	N: 35	%	N: 28	%	N: 26	%	N: 46	%	N: 38	%	N: 30	%	N:43	%
In(2L)22D;34A	4	8.6	6	21.4	0		5	10.9	2	5.3	10	33.3	11	25.6
In(2R)52A;56F	1	2.9	0		1	3.8	0		2	5.3	0		3	7.0
In(3L)63C;72F	0		0		0		2	4.6	3	7.9	0		0	
In(3L)66C;73A	0		0		1	3.8	2	4.6	2	5.3	1	3.3	0	
In(3R)89D;96A	1	5.7	0		0		2	4.6	0		0		5	11.6
In(2L)22D;34A In(2R)52A;56F	1	2.9	0		0		0		1	2.6	5	16.7	3	7.0
In(2L)22D;34A In(3L)66C;73A	0		0		0		0		2	5.3	0		0	
In(2L)22D;34A In(3L)66C;73A	0		0		0		0		0		0		0	
In(2L)22D;34A In(3R)89D;96A	0		2	7.1	0		0		0		0		1	2.3
In(2R)52A;56F In(3R)89D;96A	2	5.7	0		0		0		0		0		2	4.7
In(2L)22D;34A In(2R)52A;56F In(3L)66C;73A	0		0		0		0		0		1	3.3	0	
Heterokaryotypes	9	25.7	8	28.6	2	7.7	11	23.9	12	31.6	17	56.7	25	58.1
Homokaryotypes	26	74.3	20	71.4	24	92.3	35	76.1	26	68.4	13	43.3	18	41.9

The total frequencies of heterokaryotypes varied from 7.7% (MG) up to 56.7% (RS). Individuals with the *In(2L)22D;34A* in heterozygosis were found in all the seven strains, except MG. Heterokaryotypes for *In(2R) 52A;56F* was found in MA, MG, PR and RS strains; the heterokaryotypes for *In(3L)66C;73A* was found in SP, PR, SC and RS (all strains from southeast or south of Brazil) but, in all of them, the frequencies were not higher than 8%. Heterokaryotypes for *In(3R)89D;96A* was found in three strains (MA, SP and RS) and for *In(3L)63C;72F* only in two (SP and PR). The frequencies of these two heterokaryotypes was also low, except in RS strains, in which the frequency of *In(3R)89D;96A* was 11.6% (Table 1). Five strains presented individuals with more

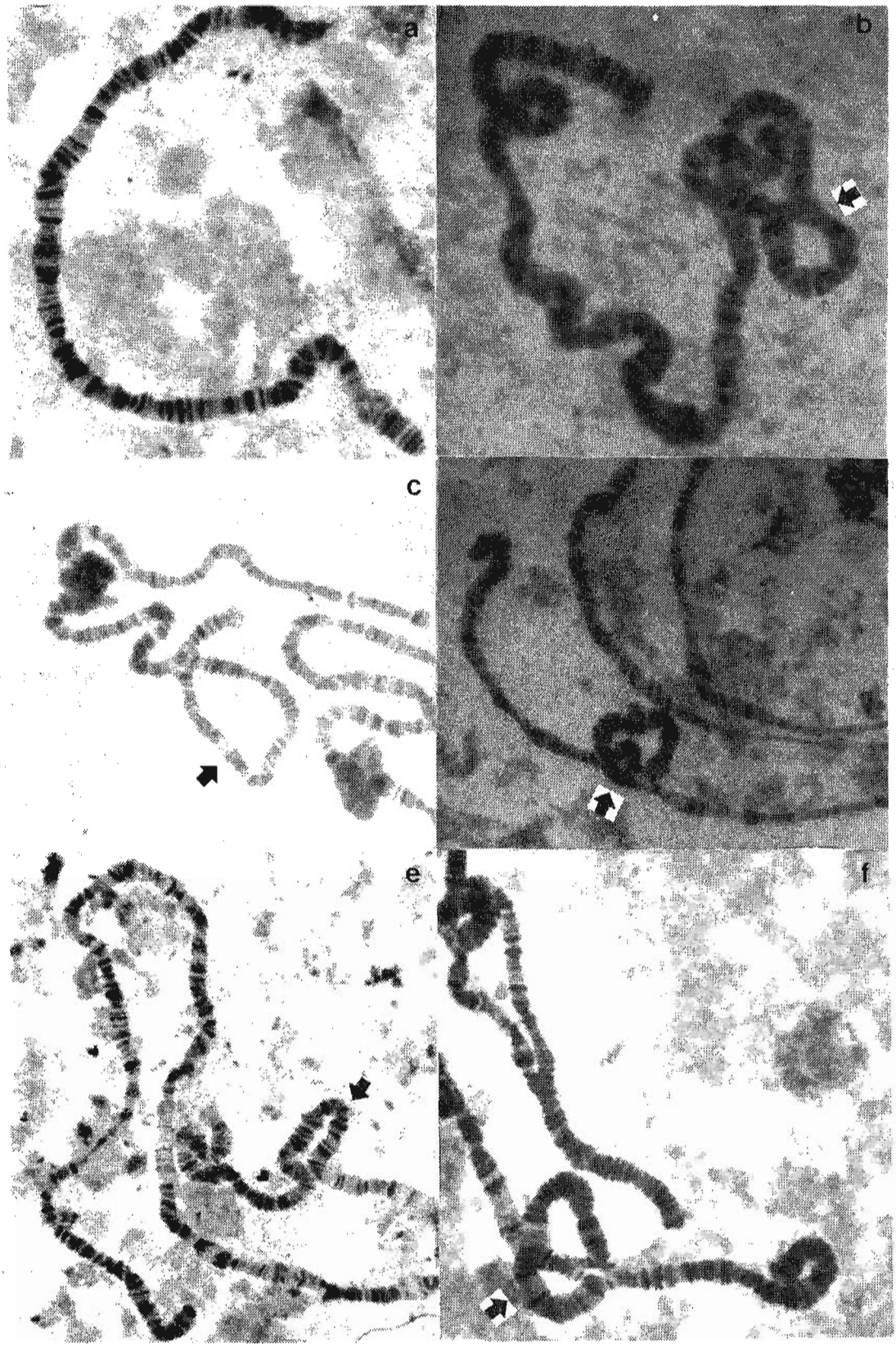

Figure 1. Cosmopolitan inversions: b. *In(2R)52A;56F,* c. *In(2L)22D;34A*, d. *In(3L)63C;72F*, e. *In(3L)66C;73A*, f. *In(3R)89D;96A*. There were not found inversions in chromosome X (a).

than one gene arrangement in heterozygosis (MA: 33.3%; PI: 25%; PR: 25%; SC: 35,3% and RS: 24%). Most of these two inversion combinations contain the *In(2L)22D;34A* gene arrangement.

Discussion

All the inversions found are cosmopolitan, common or rare (Lemeunier and Aulard, 1992). With the exception of strain MG, after at least two years of maintenance in laboratory, and thus for at least 50 generations under standard conditions at 25°C, the heterokaryotypes persisted at moderate (23.9 to 31.6%) to significantly high (56.7 and 58.1) rates. As there is no data from the original populations from which these strains derived, neither the laboratory stocks in the early generations, it is impossible to decide which strains is highly polymorphic.

The high frequency of polymorphism shown in this study results from the presence of *In(2L)22D;34A/ST* karyotype. Alone, or in combination with the others, it frequencies is over 10% in all strains (except in MG strains were is absent). The frequency this heterokaryotype in SC strain is as high as 53.3%. Persistence of inversions at considerable frequencies suggests that they confer some adaptive advantage. Alternatively, we could consider that they were neutral in our laboratory conditions. As *In(2L)22D;34A* is maintained at high frequencies in strains set up with flies from different populations this hypothesis seems less probable.

Since *D. melanogaster* is considered as having a flexible polymorphism and passable of loosing inversions when cultured in laboratory (Watanabe and Watanabe, 1977; Inoue, 1979), the data shows the unexpected persistence of *In(2L)22D;34A/ST* heterokaryotype in different laboratory strains. Two common cosmopolitan inversions, *In(2L)t* and *In(3R)P*, shown to be eliminated from cage populations by Inoue (1979), were observed in our strains, *In(2L)22D;34A* and *In(3R)89D;96A*, respectively.

In(2L)t is a very common cosmopolitan inversion that provides a well documented example of action of natural selection in maintenance of polymorphisms. Knibb (1982), from a collections from Australasia, North America and Asia-Europe published in 22 reports found latitudinal clines in the frequency of this inversion, with negative correlation of frequency against latitude. But the precise relationship between frequency and latitude varies between regions and the cline was not observed in all three regions. Bellow 30°'Slat, the frequencies of *In(2L)t* is 18.7% in North America and as high as 55.3% in Asia.

It has been shown that heterokaryotype superiority is responsible for the worldwide latitudinal distribution and seasonal fluctuation of *In(2L)t* frequencies (Van Delden and Kamping, 1989,1991; Kamping and Van Delden, 1999a,b). In cage populations, initiated polymorphic for *In(2L)t* and maintained at 20°C and 25°C, a significant decline of frequencies was observed. At 29°C and 33°C there was no change in *In(2L)t* frequencies but a significant excess of heterokaryotypes occurred. These results seems to be contrasting with those presented in this report.

There are few similar reports for the maintenance for inversion polymorphism in laboratory strains of *D. melanogaster*. Das and Singh (1990) studying *D. melanogaster* populations from India also reported that the both *In(2L)t* and *In(3R)P* were maintained in laboratory standard conditions for more than 20 months, at frequencies varying from13.73% to 82.84%. Also Santos and Valente (1991) screening stocks of this species maintained 18 years under laboratory conditions found high frequency of cosmopolite paracentric inversions which frequencies varied from 7% to 25%. The authors also found the arrangement *In(3R)89D;96A* in high in a laboratory stock from Petrópolis (a city very close to Porto Alegre, from where RS strain come from). The possible factor responsible for the high polymorphism in laboratory stocks advanced was proposed to be the superiority of

heterozygotes. The richness of culture medium and weak competition for space and food was considered the environmental factors favoring the heterozygotes.

Alahiotis *et al.* (1977) studying the selective effect of medium and humidity in cage populations reported that medium appears to influence allele and inversion frequencies while humidity does not. These authors speculate that *In(2L)t* is advantageous in environments with high population densities. According Lemeunier and Aulard (1992) this could explain the increase of frequency of *In(2L)t* observed by Roca et al. (1982) in cage population experiments. Also could explain the high frequency of heterokaryotypes for this inversion in some of our strains, since populations were maintained in bottles with high density.

At the present, it is not completely clear which could be the major balancing selection mechanism responsible for the maintenance of inversion polymorphism in *Drosophila.* Supergene selection can be potentially important mechanism of balancing selection (Alvarez and Zapata, 1997). When both supergene and karyotype selection is simultaneously operating on two gene arrangements, the recombination effect will lead to a protected polymorphism in many instances, even when the karyotype viability tends to produce the fixation of one of the two arrangements. These processes and the observation that *In(2l)t* heterozygotes exhibit heterosis with respect to certain components of fitness (Van Delden and Kamping, 1989,1991; Kamping and Van Delden, 1999a,b) could explain the persistence of *In(2L)22D;34A/ST* heterokaryotype in our stocks.

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A_{2+8+9}: a unique and new complex chromosomal gene arrangement in *Drosophila subobscura.*

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The species *Drosophila subobscura* displays a rich chromosomal inversion polymorphism on all of its five acrocentric chromosomes. Until 1993, 67 different inversions in 93 gene arrangements