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RELEASE OF VARIABILITY THROUGH RECOMBINATION OF *Drosophila melanogaster* X CHROMOSOMES, WITH SEX-LIMITED EFFECT

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ABSTRACT

Synthetic effects released through recombination were investigated for X chromosomes of normal viability, of *Drosophila melanogaster* from four localities in Brazil. The normal viability chromosomes and the recombinants were obtained in crosses sequence with the Muller-5 strain.

Evidence is presented for a relevant role of X chromosomes recombination releasing genetic variability with detrimental effects on viability, limited to the female sex. Synthetic semilethals and subvital chromosomes amounted to 52% of the 138 recombinants analyzed.

INTRODUCTION

The effect of homozygosis of recombinants of normal viability chromosomes was first shown by Dobzhansky (1946), studying chromosomes II of *Drosophila pseudoobscura.* The variability released through recombination, named "synthetic", had effects on time of development, on morphological traits and on viability of the flies, this ranging from lethality to supervitality.

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Analogous to the synthetic lethality was the synthetic sterility effect shown by Krimbas (1960, 1961) in autosomes of *D. willistoni*. Synthetic lethals were studied also in autosomes by Spassky *et al.* (1958) in *D. pseudo-obscura*, Spiess (1959) in *D. persimilis* and Dobzhansky *et al.* (1959) in *D. prosaltans*. Synthetic lethals were detected in autosomes of *D. melanogaster* by Misro (1949), Wallace *et al.* (1953), Gibson and Thoday (1962), Kosuda and Moriwaki (1968) and Malajovich (1979) but not by Hildreth (1956), Gantner (1958) and Spiess and Allen (1961). Autosomal effects are similar in both sexes.

Hildreth (1955) has investigated ten wild strains of *D. melanogaster*, concluding for a nonsignificant role of recombination of X chromosomes in producing synthetic lethality.

A concealed variability in X chromosomes of *D. melanogaster*, with sex limited effect was evinced by Kerr and Kerr (1952). The viability of homozygous females tends to be lower than that of hemizygous males or females carrying two different X chromosomes. As a measurement of the sex limited effect the proportions of females and males are used. Similar results were obtained by Drescher (1964), Gallo (1970, 1978), Gallo and Salceda (1974) and Renesto (1977). Two lethal genes with feminine sex-limited effect were detected by Drescher (1964) and Gallo and Salceda (1974) in natural populations of *D. melanogaster*.

Gene effects limited to the female sex were reported for Hymenoptera populations, particularly the Apidae, by Kerr (1951), Kerr and Laidlaw (1956), Rothenbuhler *et al.* (1968), Kerr (1969a,b).

This is an investigation of synthetic sex limited effects in normal X chromosomes of *D. melanogaster* from different localities.

MATERIALS AND METHODS

The experiment was carried out with strains derived from recent collections of *D. melanogaster* in three localities in the State of São Paulo (São José do Rio Preto, Olímpia and Pirassununga) and one in the State of Rio Grande do Sul (Porto Alegre). The symbols RP, OL, PI and PA, respectively, will be used in text and Tables. The well known Basc or Muller-5 strain (Lindsley and Grell, 1967) was also used.

The hemi-homozygosis of the X chromosomes was obtained through the following sequence of crosses:

P	RP_1/RP_2	x	M-5/Y
F_1	RP_1/M-5	x	M-5/Y
F_2	RP_1/M-5	x	RP_1/Y
F_3	RP_1/RP_1 + RP_1/Y + RP_1/M-5 + M-5/Y		

Chain crosses with heterozygous F_2 flies from the different localities (RP_1/M-5 x OL_1/Y; OL_1/M-5 x PI_1/Y and so on) were used as control.

The recombinants were obtained by intercrossing strains of normal viability, using one strain per locality. The normal viability corresponds to a sex ratio nonsignificantly different from the control sex ratio. Sex ratio is expressed as females/100 males. With F_3 flies from the chosen strains six sequences of crosses were continued with the following pairs: RP/RP x OL/Y, RP/RP x PI/Y, RP/RP x PA/Y, PI/PI x OL/Y, PA/PA x OL/Y and PA/PA x PI/Y. One of the sequences is illustrated below.

F_3	RP/RP	x	OL/Y
F_4	RP/OL	x	M-5/Y
F_5	RP-OL/M-5	x	M-5/Y
F_6	RP-OL/M-5	x	RP-OL/Y
F_7	RP-OL/RP-OL +RP-OL/Y+ RP-OL/M-5 + M-5/Y		

From F_5 on, several pair-mating crosses were performed, leading to the hemi-homozygosis of different recombinants.

All crosses were made in vials with banana-agar medium with two transfers every 48 hours and maintained at 25°C.

RESULTS AND DISCUSSION

Experiment control

The 26 crosses comprising the control chain yielded 1224 males and 1316 females, with a sex ratio of 107.51 females to 100 males, which does not differ significantly from 106.87 (L' Hèritier and Teissier, 1936), 104.81 (Kerr and Kerr, 1952), 110.08 (Gallo, 1978) and 105.17 (Gallo and Salceda, 1978). The four chi square values are lower than 3.84 (P = 0.05).

Feminine sex limited effect

Table I gives the totals of males and females yielded in F_3 of the crosses from each locality. The sex ratios of the four localities are significantly lower than the control (107.51), as indicated by the chi square values. This means a sex limited effect, detrimental for females. There is no significant heterogeneity among the ratios of the four localities ($\chi^2 = 0.22, P > 0.05$, 3 df).

The frequencies of semilethal and quasi-normal (subvital, normal and supervital) chromosomes for the four localities are presented in Table II. Out of the 77 chromosomes analyzed, 27 (35%) exhibited semilethal or subvital effects on female viability.

Table I -Number of males and females in F_3 hemi- and homozygotes for different wild X chromosomes.

Strains	Chromosomes analyzed	F_3			χ^2
		Males	Females	Sex ratio	
RP	18	1168	1113	95.30	8.30**
OL	21	950	927	97.60	4.42*
PI	17	726	698	96.14	4.44*
PA	21	881	837	95.00	6.57**

χ^2_1 for the 107.51 control sex ratio, *: $P < 0.05$, **: $P < 0.01$.

Table II - Distribution of the chromosomes from the four localities in classes of viability, according to the F_3 sex ratios.

Viability class	RP		OL		PI		PA		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Semilethal	–	–	2	9.52	2	11.76	1	4.77	5	6.50
Subvital	7	38.89	4	19.04	5	29.41	6	2.86	22	28.56
Normal	8	44.44	10	47.62	6	35.29	12	57.14	36	46.74
Supervital	3	16.67	5	23.81	4	23.52	2	9.52	14	18.20
Total	18		21		17		21		77	

Feminine sex-limited effect released by recombination

Table III presents the phenotype frequencies in F_3 for the normal chromosomes used in the recombination tests, whose progenies in F_7 are shown in Table IV. The sex ratio for the six combinations are all significantly lower than the 107.51, indicating detrimental effects on female viability. The mean of the sex ratios is 90.27, also significantly lower than 107.51, indicating detrimental effects on female viability ($\chi^2 = 122.25$, $P < 0.001$, 1 df).

The distribution of the recombinant chromosomes in viability classes is given in Table V. Table VI gives the phenotype frequencies and sex ratio of the 14 semilethal chromosomes detected: 5 in the extraction of the wild chromosomes (F_3) and 9 resulting from recombination (F_7).

The same question was studied by Hildreth (1955), for X chromosomes of *D. melanogaster* affecting both sexes which were carriers of the same chromosome. His results indicated an irrelevant role of X chromosome recombination producing lethal effects. Dobzhansky *et al.* (1942) have already called attention to the fact that "owing to the frequent passage of the X chromosome through the hemizygous state in the male, sex-linked mutants which lower the adaptive level of the organism in its normal environment must be eliminated relatively promptly. Only neutral variants or genetic factors which express themselves differently in the two sexes could accumulate in the X chromosomes". This is corroborated by the two X chromosomes found by Drescher (1964) and Gallo and Salceda (1974) with lethal effect on females but not on males; this is a very low frequency for all the X chromosomes studied by different authors.

Table III -Phenotype frequencies in F_3 of the normal chromosomes used in recombination tests.

No. of chromosomes	Strains	Marked flies		Wild flies			
		M-5/Y	X/M-5	X/Y	X/X	Sex ratio	χ^2
12	RP	33	53	46	49	106.52	0.00
9	OL	24	33	38	39	102.63	0.04
14	PI	51	65	75	76	101.33	0.13
19	PA	57	52	82	96	117.07	0.32

χ_1^2 for the 107.51 control sex ratio.

Table IV -Totals of males and females in F_7 hemi-and homozygotes for different recombinant X chromosomes.

Type of recombinant	Chromosomes analyzed	F_7			χ^2
		Males	Females	Sex ratio	
RP – OL	29	1478	1160	78.48	64.89**
RP – PI	20	1533	1492	97.32	7.49**
RP – PA	23	1219	1105	90.65	16.90**
OL – PI	17	1027	992	96.60	5.80*
OL – PA	35	2003	1814	90.56	28.07**
PI – PA	14	990	871	88.00	18.68**

χ^2_1 for the 107.51 control sex ratio; *: $P < 0.05$, **: $P < 0.01$.

Spassky *et al.* (1958) considered that the irrelevance of X chromosomes recombination with lethal effect "is not a very remarkable result since one third of the X chromosomes in a population are carried in males which would die if they had a sex-linked lethal. Therefore, X chromosomes capable of giving sex-linked lethals by recombination with other X's in the same population would be discriminated against by natural selection. . . . Only synthetic lethals that would kill homozygous females, but not the males, are expected in the X chromosomes".

The lethality of synthetic systems, according to Magalhães *et al.* (1965), is only a negative feature of a coadapted system with a positive adaptive value, important in the maintenance of the natural populations variability. Although no lethals have been observed, a very high frequency of recombinants with detrimental effect on female viability was observed: 72 (52%) out of the 138 chromosomes analyzed were semilethal or subvital. This may be taken as evidence for a relevant role of X chromosomes recombination in producing detrimental (semilethal and subvital) synthetic effects, limited to females.

Table V - Distribution of the different types of recombinants in classes of viability, according to the F_7 sex ratios.

Viability class	RP - OL		RP - PI		RP - PA		PI - OL		PA - OL		PA - PI		Total	
	No.	%	No.	%	No.	%	No	%	No.	%	No.	%	No.	%
Semilethal	2	6.89	–	–	3	13.04	–	–	3	8.57	1	7.14	9	6.52
Subvital	18	62.06	6	30.00	8	34.78	6	35.29	18	51.42	7	50.00	63	45.65
Normal	7	24.13	14	70.00	11	47.82	7	41.18	9	25.71	5	35.71	53	38.40
Supervital	2	6.89	–	-	1	4.34	4	23.53	5	14.29	1	7.14	13	9.42
Total	29		20		23		17		35		14		138	

Table VI -Phenotype frequencies of the wild (F_3) and recombinant (F_7) semilethal chromosomes.

No. of the chromosomes	Strain	Marked flies		Wild flies		
		M-5/Y	X/M-5	X/Y	X/X	Sex ratio
WILD						
8	OL	23	23	32	15	46.88
17	OL	22	41	47	23	48.89
4	PI	19	18	22	9	40.91
15	PI	10	16	23	10	43.48
2	PA	17	18	29	13	44.83
RECOMBINANT						
8	RP – OL	38	45	45	22	48.89
35	RP – OL	88	107	100	5	5.00
2	RP – PA	10	22	24	8	33.33
25	RP – PA	14	21	25	9	36.00
26	RP – PA	17	22	27	12	44.44
1	PA – OL	5	18	22	9	40.91
13	PA – OL	12	13	22	11	50.00
15	PA – OL	25	30	48	23	47.92
9	PA – PI	46	32	74	33	44.59

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RESUMO

Cento e trinta e oito combinações de cromossomos X de *Drosophila melanogaster* foram estudadas para o efeito limitado ao sexo feminino, por recombinação de cromossomos de viabilidade normal, de quatro estoques selvagens, provenientes de quatro localidades (três de São Paulo e uma do Rio Grande do Sul).

Os resultados demonstram a existência do efeito limitado ao sexo, com liberação de variabilidade oculta, indo da semiletalidade à supervitalidade.

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