

# Size polymorphism of the X-chromosome due to attachment of a B-chromosome in the Medfly, *Ceratitis capitata* (Wied.)

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## ABSTRACT

A chromosomal polymorphism affecting the length of the long arm of the X-chromosome, along with the presence of a B-chromosome is reported in a laboratory population of *Ceratitis capitata*. The observed B-chromosome is small, heterochromatic and telocentric. It was found, in both sexes, in somatic cells (cerebral ganglia tissue) as a free chromosome, or terminally attached to the long arm of the X-chromosome, giving the appearance of a larger X chromosome. Males transmit both free B and large X-chromosomes to their progeny. Females only transmit large X to their progeny, at a higher rate than they do the standard X, suggesting either a differential utilization of the ovules carrying the large X or a preferential coorientation during the first meiotic division.

## INTRODUCTION

Studies carried out in our laboratory, have shown reproductive isolation between laboratory and field populations of the Mediterranean fruitfly (Medfly) *Ceratitis capitata* (Martinez *et al.*, 1988). The normal chromosome complement of the medfly has a diploid number of 12. It includes two pairs of submetacentric and three pairs of metacentric autosomes, while the X and the Y-chromosomes are acrocentric (Lifschitz and Cladera, 1989).

Descriptions of variations in chromosome morphology in the Medfly are scant; only three reports exist. Gasperi *et al.* (1983) informs about the occurrence of a satellite in the long arm of the Y-chromosome in some Italian populations of the insect. Manso and Lifschitz (1986) found a B-chromosome in a field population; they

also describe a variant affecting the centromere size of chromosome 3. Cladera *et al.* (1987) refers to a small submetacentric Y-chromosome in a laboratory strain.

## MATERIAL AND METHODS

### Material

The flies used in the present study were from a 12 year old laboratory population named ARG 51 at Castelar. This stock was maintained because a polymorphism for the length of the X-chromosome had been detected in a screening of laboratory material.

### Preliminary procedures

A sample of 300 larva taken from stock 51 were examined. To increase the frequency of the large X-chromosome, flies from stock 51 were single pair mated for six generations.

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The progeny of crosses exhibiting a high frequency of the large X-chromosome were chosen as parents of the next generation. Then the family with the highest frequency of large X was mass mated for another four generations. The resulting strain was designated as 12986.

From here on, the normal size X-chromosome will be named  $X_S$  and the large one  $X_L$ .

The Castelar line ARG 17, free of  $X_L$ , was the standard strain employed for the test crosses.

All experiments were carried out under laboratory conditions, 23-27°C and 65-80% RH.

## Methods

Mitotic metaphase plates were prepared from neuroblast cells of third instar larvae. Reproductive tissue was obtained from pupae one day before adult emergence. Meiosis could be studied in primary and secondary spermatocytes of males, but in females only mitotic metaphases of oogonia and follicular cells were observed. In both cases the tissue was stained in 4% orcein solution.

Slides were obtained by squashing the tissue in lacto-propionic medium (1:1). The presence of either  $X_L$  or B chromosomes was recorded only after positive identification in at least 10 mitotic or meiotic plates.

## Experiment I

Before establishing strain 12986, a study was performed on "stock 51" using an index based on the ratio between the sex chromosomes present in the same metaphase plate. This value was obtained in the male karyotype as a ratio between the length of Y/X and in the female karyotype, the ratio was  $X_S/X_L$ .

## Experiment II

The aforementioned index obviously did not distinguish between  $X_L/X_L$  and  $X_S/X_S$  individuals. For that purpose, a different index was computed. It was a ratio between the length of the short and the long arm of the X-chromosomes.

## Experiment III

To ascertain the frequency of  $X_L$  in strain 12986, a sample of 400 larvae was examined; ten metaphases of each individual were studied.

## Experiment IV

The mode of inheritance of the  $X_L$  and B-chromosomes was studied by performing 61 reciprocal single pair matings between stock 12986 and line ARG 17; ten larvae of each cross were studied. The X-chromosomes were classified according to the index calculated in "Experiment II".

## RESULTS

Unlike the autosomes, the sex chromosomes rarely appear as somatic bivalents. It was under this infrequent configuration that female heterozygote karyotypes  $X_S/X_L$  were initially detected in larvae from stock 51. As only the long arm was affected, the X-chromosome did not lose its acrocentric characteristic (Figure 1).

## Experiment I

The distribution of indexes for polymorphic stock 51 and line ARG 17 are shown in Figure 2. These data are normally distributed.

The distribution of the index X/X is shown in Figure 2A. For stock 51 the mean was  $0.86 \pm 0.027$  and for line ARG 17 it was  $0.95 \pm 0.018$ , the difference between them being highly significant ( $P < 0.01$ ). The variances were also statistically different, being larger for stock 51 ( $P < 0.01$ ).

The distribution of the index Y/X is shown in Figure 2B. Again, two populations of data showing statistically different means and variances ( $P < 0.01$ ) were obtained. For stock 51 the mean was  $0.55 \pm 0.025$  and for line ARG 17 it was  $0.62 \pm 0.019$ .

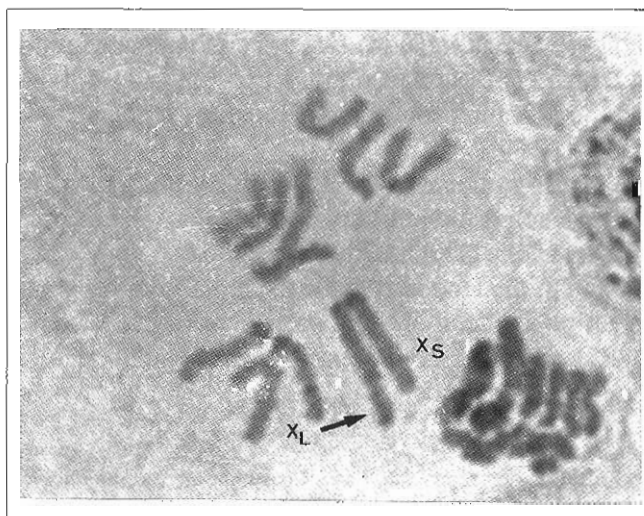


Figure 1 - Ganglia metaphase with the large X-chromosome indicated by an arrow.

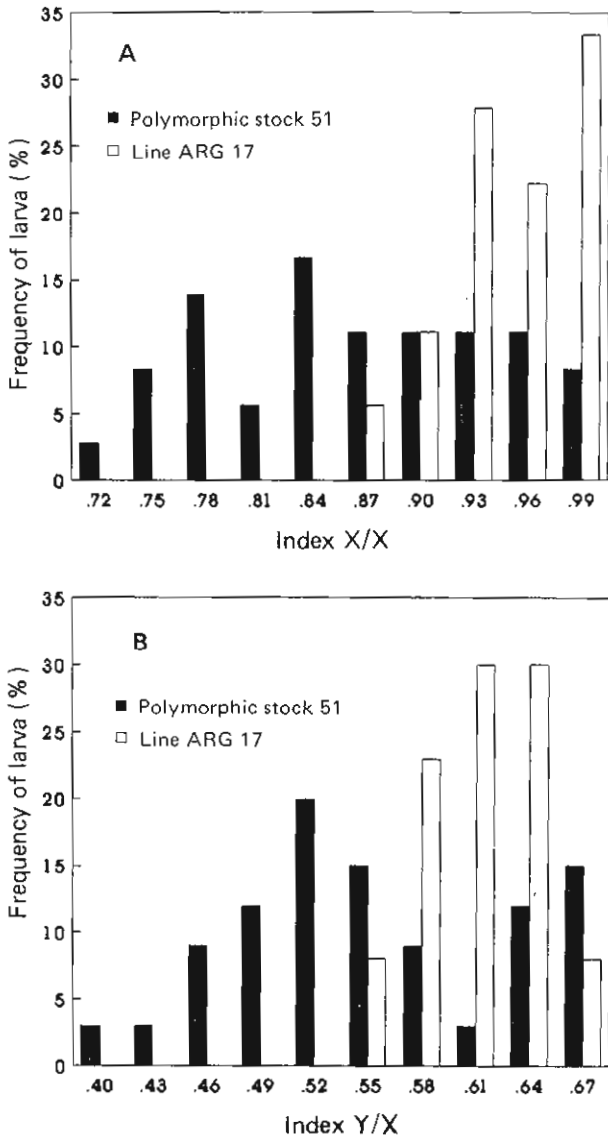


Figure 2 - Index distribution for the polymorphic stock 51 and line ARG 17. A: X/X. B: Y/X.

**Experiment II**

The mean ratios between the short and the long arm for the  $X_L$  ( $0.15 \pm 0.012$ ) and  $X_S$  ( $0.19 \pm 0.007$ ) chromosomes from heterozygous individuals of stock 51 were statistically different ( $P < 0.01$ ). The ratio computed for  $X_S$  in heterozygous individuals of stock 51 was not different from the ratio computed for the control line ARG 17.

**Experiment III**

Stock 12986 had four different types of karyotypes (Table I). The B-chromosome present was small, one-fifth the size of the standard X-chromosome, telocentric and generally heteropycnotic (Figure 3).

Table I - Frequency of individuals bearing polymorphic karyotypes in Medfly Strain 12986 (n = 400).

	Type of metaphase		
	All metaphases with B chromosomes	All metaphases with $X_L$ chromosomes	Metaphases with B or $X_L$ in the same individual
Normal	11%	29%	28%
			32%



Figure 3 - Ganglia metaphase with a free B chromosome indicated.

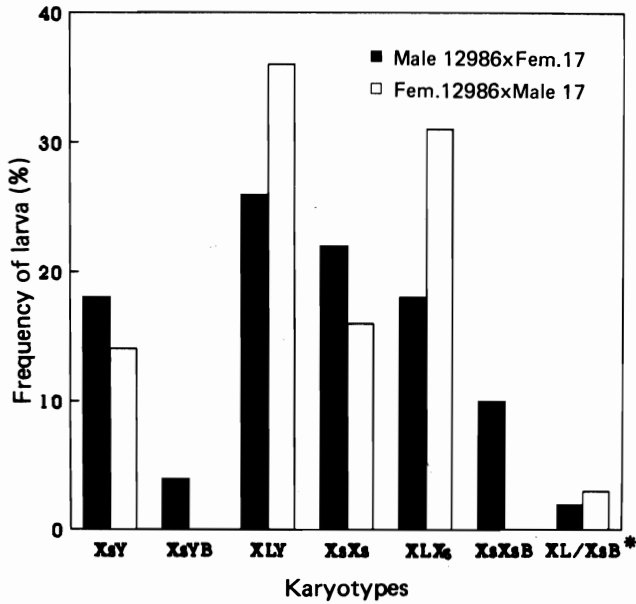
Assuming random mating, in a sample of 400 larvae, 89% of them with either  $X_L$  or B chromosomes present, the detection of homozygous  $X_L/X_L$  individuals would have been expected. However, such individuals were not observed in this sample.

**Experiment IV**

*Mitotic analysis*

A total of 610 larvae were studied. Among them, 130 showed the normal karyotype and 480 carried either the  $X_L$  or the B-chromosome. In Figure 4 the segregations of the different karyotypes of the progeny are shown.

The progeny of stock 12986 females did not show free B-chromosomes but had the  $X_L$ -chromosome at a high rate. In fact, 67% of the progeny carried  $X_L$  ( $X_L/X_S + X_L/Y$ ) and 30%  $X_S$  ( $X_S/X_S + X_S/Y$ ). Three percent of the larvae, showed  $X_L/X_S$  and  $X_S/X_SB$  metaphase plates in the same individual.



**Figure 4** - Histogram of the different karyotype segregations resulting from reciprocal single pair crossings between flies 12986 and ARG 17. \*In the same individual, some were X<sub>L</sub>/X<sub>s</sub> metaphases and others X<sub>s</sub>/X<sub>s</sub>B.

In the progeny of stock 12986 males, not only X<sub>L</sub>-chromosomes but also free B-chromosomes were found. According to Figure 4, 14% of the offspring showed free B-chromosomes: 4% X<sub>s</sub>YB and 10% X<sub>s</sub>/X<sub>s</sub>B. They also showed X<sub>s</sub> and X<sub>L</sub> at similar

frequencies: 22% X<sub>s</sub>/X<sub>s</sub> and 18% X<sub>L</sub>/X<sub>s</sub>. As in female progenies, a small proportion (2%) of larvae showed mixed metaphase plates. The remaining 44% was distributed as follows: 26% X<sub>L</sub>/Y and 18% X<sub>s</sub>/Y.

As the occurrence of the X<sub>L</sub>/Y karyotype in the progenies from crosses between males 12986 by females ARG 17 was unexpected, an analysis of the possible gametes produced by the father was performed; its results are shown in Table II.

Studying separately the contribution of X and Y gametes to the male gametic pool, a balance among those carrying the X-chromosome was observed. But when analyzing those carrying the Y-chromosome a strong unbalance was found: 44% Y (X<sub>L</sub>/Y and X<sub>s</sub>/Y) and 4% YB (X<sub>s</sub>YB) (Table II).

A priori, observing Figure 4, an X<sub>L</sub>/Y son would arise from an X<sub>L</sub>/Y father and an X<sub>s</sub>YB son would arise from an X<sub>s</sub>YB father. However, both X<sub>L</sub>/Y and X<sub>s</sub>YB sons arose necessarily from a male gamete carrying **Y and B**. If a male produced a son, then the male was transmitting the Y-chromosome, which is male determining (Lifschitz and Cladera, 1989). Besides, the son's mother was wild type, so she was transmitting an X<sub>s</sub> chromosome. Consequently, a proportion of males showed X<sub>L</sub>-chromosomes, which must have received them as free B-chromosomes. Therefore, stock 12986 males have segregated Y gametes and YB gametes (Table II).

**Table II** - Frequencies of the different karyotype segregations. Analysis of the possible gamete produced by the father in the crossing male 12986 x female ARG 17 (from Figure 4).

Larval genotypes (%)	Possible genotype of male gamete from which larvae arose (%)		
	A priori	Gamete pool	X and Y gametes separately
X <sub>s</sub> Y - 18%	44% Y	<b>Y 18%</b>	38%
X <sub>L</sub> Y - 26%			
X <sub>s</sub> YB - 4%	4% YB	<b>YB 30%</b>	62%
X <sub>s</sub> X <sub>s</sub> B - 10%	Free B* - 14%		
X <sub>s</sub> X <sub>s</sub> - 22%		<b>Free B** - 40%</b>	20%
X <sub>L</sub> X <sub>s</sub> - 18%			
		<b>X<sub>s</sub>B 10%</b>	20%
		<b>X<sub>s</sub> 22%</b>	44%
		<b>X<sub>L</sub> 18%</b>	36%

\*Observed in larvae.

\*\*Transmitted as free B but observed as free B or as X<sub>L</sub>.

### Meiotic behaviour of $X_L$ and B-chromosomes

In spermatocytes the B-chromosomes appear as a small telocentric and heteropycnotic chromosome, sometimes free (Figure 5), or attached end to end to the X-chromosomes (Figure 6). Cells with 0B or 1B, in the same testes, were also observed. In females, cells with  $X_L$  and  $X_S$  in the oogonia were observed, but free B-chromosomes were not found (Figure 7).

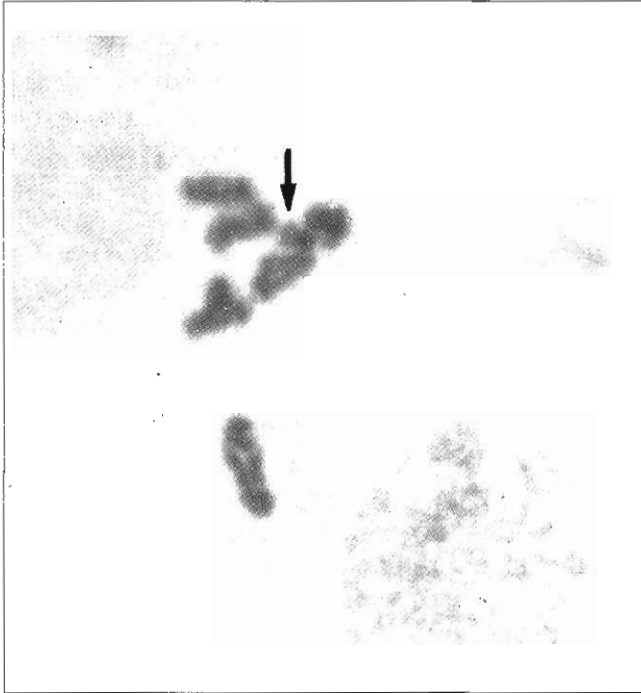


Figure 5 - Primary spermatocyte metaphase showing a free B-chromosome.

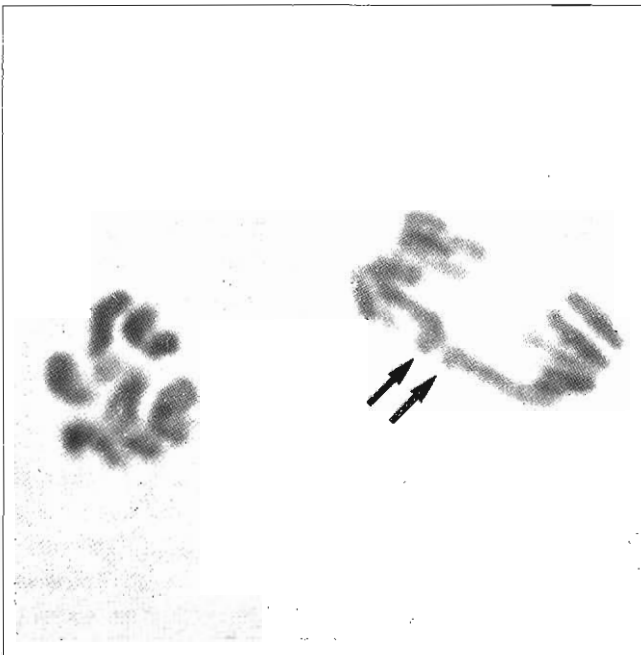


Figure 6 - Secondary spermatocyte. Arrow indicates the B-chromosome attached end to end to the X-chromosome.

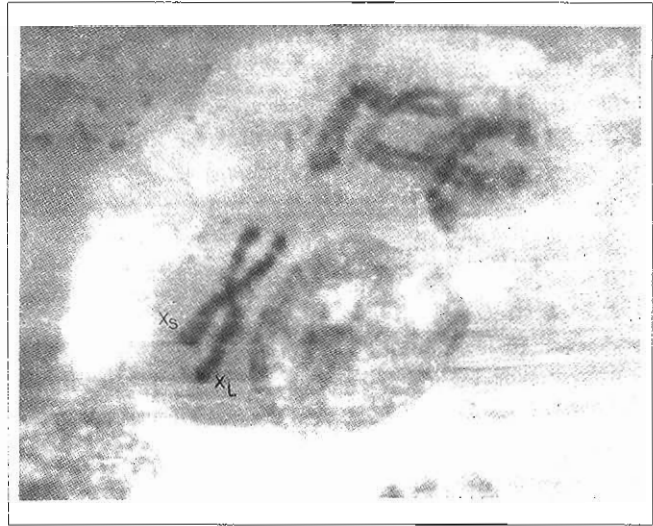


Figure 7 - Sexual pair of oogonia.

## DISCUSSION

Among 3550 metaphases, the  $X_L$  and B-chromosomes were never present simultaneously in the same metaphase plate. The mutually exclusive presence of the  $X_L$  and B chromosomes and the results of male offspring data indicating that  $X_L Y$  eggs are produced by YB gametes (Figure 4), suggest that the  $X_L$  chromosome arises when the B is attached terminally to the long arm of an  $X_S$ . This can explain the unexpected presence of the  $X_L Y$  karyotype in crosses between the polymorphic males and normal females (Figure 4). In this case, males are transmitting YB gametes and in the zygote the B-chromosome is attached end-to-end to the long arm of the  $X_S$  chromosome, coming from the female which is wild type. If so, males transmit: free B 40%,  $X_L$  18%,  $X_S$  22% and 18% Y (Table II). Therefore, unlike the females, males transmit  $X_L$  and  $X_S$  at the same frequency (non significant differences) and free  $B_S$  at a high rate. Besides, free  $B_S$  are transmitted through Y gametes (that is YB gametes) to 30% of the offspring and through  $X_S$  gametes ( $X_S B$ ) to 10%, which would have to be added to the other type of X gamete, meaning 18%  $X_L$  gametes. So, there are 30% YB plus 28%: 10%  $X_S B$  and 18%  $X_L$  (Table II); at least 40% of which are transmitted as free  $B_S$  or as  $X_L$  in the progeny. The obvious explanation is a viability difference because the male gametic pool shows the following frequencies: 58% gametes carrying free B or  $X_L$  and 40% normal  $X_S$  or Y gametes.

Although the B-chromosomes were observed in both sexes, not only in somatic tissue (neuroblasts of cerebral ganglia) but also in germ cells, the results of

the reciprocal crosses (Figure 4, Table II) strongly suggest that only the males transmit free B-chromosomes. In the Medfly it is difficult to analyze the course of gametogenesis, because in each individual all cells are synchronized in the same developmental stage. Thus the meiotic behaviour has been studied in some stages but not in others. The observation of testes cells with a seventh small bivalent (Figure 5) points out that larvae with or without B-chromosomes must be produced. In fact, males transmit the  $X_S$  chromosome to the offspring (Figure 4). The mechanism is different in females; the B-chromosomes, although present in the oogonia (Figure 7), are transmitted to the next generation, always terminally attached to the  $X_S$  chromosome (Figure 4). A high rate of  $X_L$  transmission relative to  $X_S$  was observed. This could be due either to a differential utilization of both types of ovules or to a preferential coorientation of  $X_L$  chromosomes during the first meiotic division.

The numerical variation of free or attached B-chromosomes from one follicle to another in the germ line of males (Figures 5 and 6) and females, indicates a "mitotic instability" (White, 1951) in the medfly.

Nur (1963, 1969) suggests that an intra-individual variation in the number of supernumeraries, which results from a non-disjunction, will not be maintained in the population, unless it is associated with an accumulation mechanism. It would be lost during some of the divisions prior to gamete formation. In a considerable number of living organisms the behaviour of B-chromosomes provides them with an accumulation mechanism capable of increasing their frequency in the population from generation to generation (Battaglia, 1964). In the Medfly population studied, through six generations, there is no indication that an accumulation mechanism exists, as would be suggested by the presence of the homozygote  $X_L/X_L$ . On the contrary, a mechanism which strongly inhibits the accumulation of the B-chromosome, either free or attached to the  $X_S$  must exist.

As no lagging B-chromosomes were observed; the possibility of the existence of an elimination mechanism based on this phenomenon can be discarded.

However, other explanations can be put forward. B-chromosomes are successfully transmitted to the gametes if they are incorporated undivided within a first telophase nucleus (Mendelson and Zohary, 1972). Otherwise, they would be eliminated. There is evidence, in insects, supporting this conclusion. In the grasshopper *Myrmeleotettix maculatus* the univalent B-chromosome moves undivided, in every spermatocyte, to one of the two telophase nuclei at the first division (John and Hewitt, 1965a). In the grasshopper

*Tettigidea lateralis* the incorporation of the undivided B-chromosomes into the first telophase nucleus is achieved as a result of association with an  $X_S$  chromosome at meiosis (Fontana and Vickery, 1973). Both chromosomes are associated by "sticking" of heterochromatic regions, with the result that  $X_S$  and B move to the same pole. A similar mechanism could be acting in the medfly females. The association producing the  $X_L$  chromosome seems to be very stable. On the other hand, in males, free  $B_S$  and  $X_S$  segregate at similar frequencies, indicating a weak association between both chromosomes. Probably the same mechanism discussed for the female occurs also in the male, but the association B/ $X_S$  must be dismantled at some stage during gametogenesis.

In somatic tissue the association B/ $X_S$  appears quite stable. Only a very low number of individuals, 5%, adding up the reciprocal crosses, had metaphases with  $X_L$  or B in the same ganglion in somatic tissue. A statistically significant difference in the transmission of  $X_L$  and  $X_S$  by females was observed. The high rate of  $X_L$  transmission by females could be due either to a preferential segregation into the primary oocytes or to an increased viability of the zygotes carrying  $X_L$ . In the Medfly a similar phenomenon, but involving a preferential utilization of sperm bearing  $B_S$ , was described by Manso and Lifschitz (1986). However, the mode of inheritance of the B discussed in the present paper is different; while in the former both sexes contribute B-chromosomes to the offspring, in the latter only males do so.

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## RESUMO

Foi encontrado em uma população de laboratório de *Ceratitis capitata* um polimorfismo cromossômico afetando o comprimento do braço longo do cromossomo X, junto com a presença do cromossomo B. O cromossomo B observado é pequeno, heterocromático e telocêntrico. Isto foi encontrado em ambos os sexos, em células somáticas (tecido gangliar cerebral) como um cromossomo livre, ou ligado ao braço longo do cromossomo X, aparentando um cromossomo X grande. Os machos transmitem

ambos, o cromossomo B livre e o X grande para a progênie. As fêmeas só transmitem o cromossomo X grande para a progênie e em uma taxa maior do que o X normal, sugerindo uma utilização diferencial dos óvulos que carregam o X grande ou uma coorientação preferencial durante a primeira divisão meiótica.

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