

Sex-ratio in *Drosophila mediopunctata*

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The occurrence of *sex-ratio* in *Drosophila mediopunctata* is described. The *sex-ratio* trait, affected males producing progenies with a large excess of females, is known also in eight other *Drosophila* species. It has X-linked inheritance, being apparently always associated with particular X chromosome inversions. The expression of the *sex-ratio* trait in *D. mediopunctata* is very variable.

INTRODUCTION

Sixty years ago Gershenson (1928) described “a new *sex-ratio* abnormality in *Drosophila obscura*” in which affected males had an excess of females in their progeny. This phenomenon is caused by an X-linked factor and became known as “*sex-ratio*”. Since then, it has been found in eight *Drosophila* species (see Voelker, 1972 for a list of species). In two of them the effect of the *sex-ratio* factor was investigated cytologically with similar results: the excess of females is caused by spermiogenic failure, presumably affecting the Y-bearing spermatids (Policansky and Ellison, 1970; Hauschteck-Jungen and Maurer, 1976). Hence *sex-ratio* is a special case of meiotic drive, involving the sex chromosomes.

D. mediopunctata Dobzhansky and Pavan 1943 belongs to the tripunctata group (Frota-Pessoa, 1954); it is known to occur in Brazil and El Salvador (Val *et al.*, 1981). A map of its polytene chromosomes was published in 1966 by Kastritsis, using only one isofemale line and reporting the occurrence of two gene arrangements in chromosome 2.

Since 1985 we have been studying the chromosomal inversion polymorphism of *D. mediopunctata*. We have found inversions in chromosomes X, 2, and 4 with a total of 21 gene arrangements (Klaczko and Peixoto, 1986; Peixoto and Klaczko, 1987; a detailed description of *D. mediopunctata* chromosomes will be published elsewhere). In the X chromosome four gene arrangements occur:

Standard and inversions 1, 2, and 3 (fig. 1). Inversions 1 and 2 are in strong linkage disequilibrium, usually occurring together. X chromosomes carrying both are named 2+1 or simply 21. Their frequencies in adult males are: ST: 75 per cent; 21: 10 per cent; 3: 10 per cent; 2: 3 per cent; 1: 2 per cent; $n = 849$ (Carvalho *et al.*, 1987; unpublished data). We report here that X chromosomes 2 and 21 are associated with *sex-ratio* in *D. mediopunctata*.

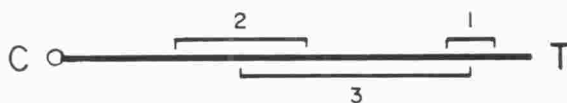


Figure 1 *Drosophila mediopunctata* X chromosome, showing the approximated positions of inversions 1, 2 and 3. C, centromere; T, telomere.

MATERIAL AND METHODS

Between September 1986 and March 1988 we made five field excursions to Parque Nacional do Itatiaia (State of Rio de Janeiro, Brazil) at intervals of approximately three months. Flies were collected mainly in early morning with fermented banana baits. After etherization, *D. mediopunctata* males were sorted from other flies under a dissecting microscope. They were crossed, in the laboratory, with three virgin females of ITC-229-ET, a homokaryotypic strain carrying the X Standard

gene arrangement. Male gene arrangements for all chromosomes were determined by the inspection of salivary gland squashes of up to eight F_1 larvae. The parents were kept at 16°C and transferred to new vials approximately every 10 days. To retard pupation half of the vials were cultured at 8°C (no directional bias in the offspring's sex-ratio is introduced by this procedure and hence the 16°C and 8°C counts were pooled). The adults emerging from one or more vials were sexed and counted until vials were exhausted. Only crosses producing 20 or more flies were considered.

For the formal genetic analysis, crosses were made in the same general scheme except that vials were always maintained at 16°C , more care was taken to avoid crowding, and another homokaryotypic strain (ITA-24-P) was used. In these crosses, male X chromosome gene arrangement was determined by inspection of one F_1 female larva (sexed after Cooper, 1950) salivary gland squash.

Flies were reared on trimeveledon, a culture medium developed by Dr A. R. Cordeiro containing integral wheat flour, yeast, milk powder and agar.

RESULTS

Field data

Fig. 2 shows the distribution of the sexual proportion (expressed as per cent of males) of progenies of wild caught males carrying different X chromosome gene arrangements. ST/Y, 1/Y and 3/Y males produced essentially normal progenies with roughly 50 per cent males; the averages were, respectively, 47.4 per cent ($n = 141$ males tested); 47.2 per cent ($n = 9$) and 46.7 per cent ($n = 25$). Not a single male among these 175 produced offspring where the proportion of males was less than 20 per cent. On the other hand 21/Y and 2/Y males were clearly abnormal producing progenies with large excess of females ("sex-ratio"). These progenies average respectively 20.5 per cent ($n = 52$) and 27.4 per cent ($n = 15$) of males, with a large variation among them. These data strongly suggest an association of inversion 2 with a factor that distorts the sexual proportion in the progeny of carrier males.

Genetic analysis

We made a formal genetic analysis of the sex-ratio trait that confirms and extends the initial conclusions (fig. 3). As X chromosome 21 is more frequent than those carrying inversion 2, the former

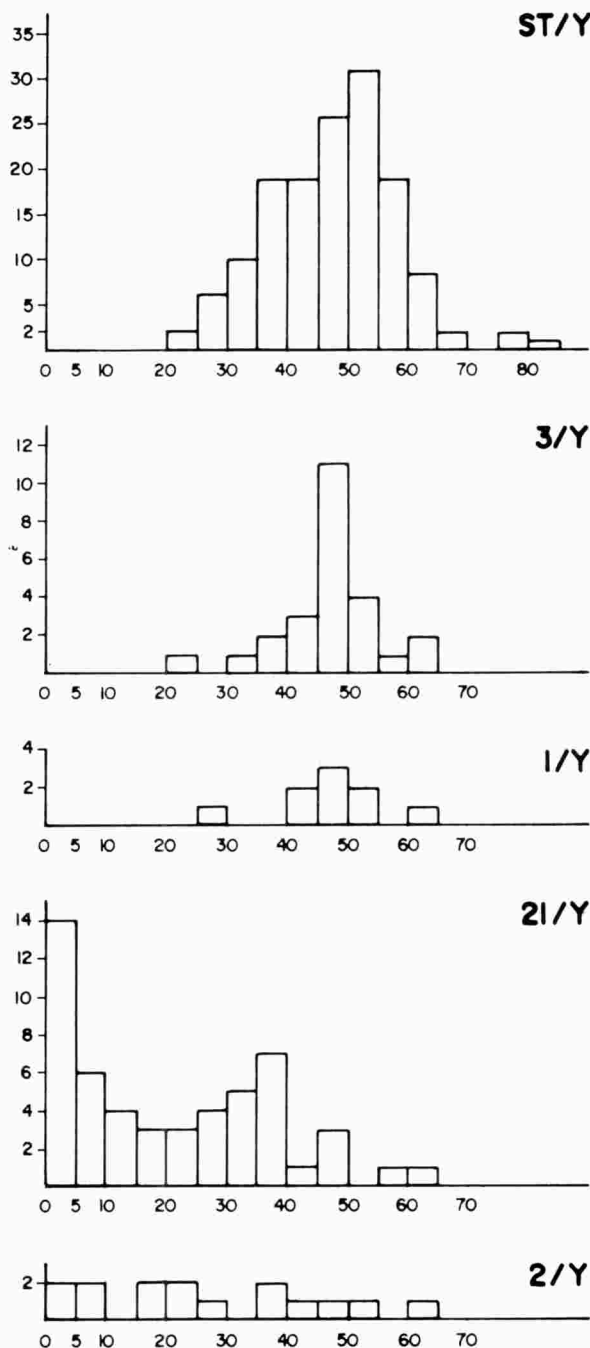


Figure 2 Progenies of wild-caught *Drosophila mediopunctata* males. Male genotype is indicated in the corresponding histogram. Abcissae, per cent males; ordinate, absolute frequency of progenies.

was used in all laboratory experiments. Five 21/Y males representing three different X:21 chromosomes were individually crossed with ST/ST females (strain ITA-24-P) and produced progenies

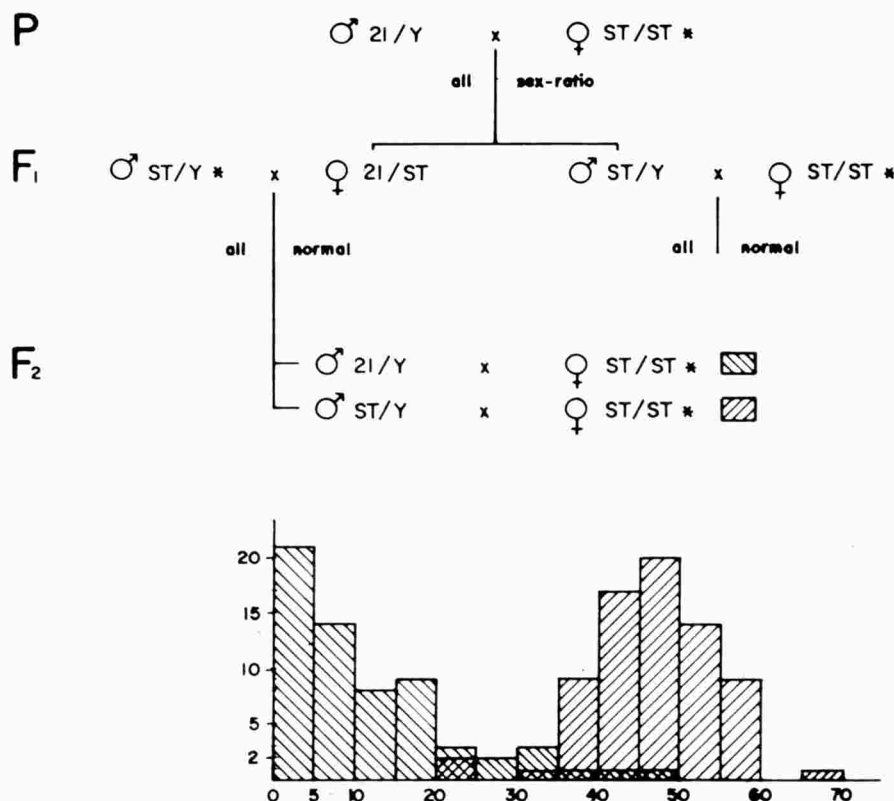


Figure 3 Genetic analysis of the sex-ratio trait. Flies marked with an asterisk were from ITA-24-P strain. Bottom histogram: progenies of F_2 males; axes are the same as fig. 2.

with a large excess of females (a total of 8 males and 184 females). F_1 males from these and equivalent crosses were fertile (41 out of 48 tested) and when pair-mated with ITA-24-P females produced normal progenies ($n=18$), as do the reciprocal crosses ($n=10$). Approximately half of the F_2 males (from the later cross; see fig. 3), those carrying the X:21 chromosome, again produced sex-ratio progenies in crosses with ITA-24-P females. Progenies of 21/Y F_2 males averaged 12.2 per cent males ($n=61$) and had a very significant heterogeneity (homogeneity chi-square = 413.7, 60 df, $P < 0.001$). Those from ST/Y F_2 males averaged 46.7 per cent males ($n=73$) and were homogeneous (homogeneity chi-square = 79.8, 72 df, $P > 0.2$). Homozygous 21/21 females produced normal progenies when crossed with ST/Y males; all F_1 males were *sex-ratio* (data not shown).

Thus the sexual proportion of a progeny is determined by the parental male genotype; only 2/Y and 21/Y males can produce *sex-ratio* progeny. The genetic analysis excludes cytoplasmic, autosomal recessive, autosomal dominant and Y-linked inheritance of *sex-ratio*'s main effect.

Recombination between inversions 1 and 2

The F_2 males in fig. 3 are sons of heterozygous 21/ST females; determining their X chromosome genotype we can estimate the recombination frequency between inversions 1 and 2. In 494 F_2 males analysed we have found 243 21/Y, 250 ST/Y and a single recombinant 1/Y giving a cross-over frequency of 0.2 per cent. We isolated the recombinant X:1 chromosome and tested ten males carrying it; none was *sex-ratio*. This shows, in accordance with the field data, that the *sex-ratio* factor is not associated with inversion 1. There is also tight linkage between the *sex-ratio* factor and chromosome X:21: we counted the progenies of more than 200 ST/Y sons of 21/ST females and none was *sex-ratio*.

Stability of the expression of the sex-ratio trait

The expression of the *sex-ratio* trait is variable. Homogeneity chi-square tests almost always show strong departures from homogeneity among the progenies of different 21/Y males. This seldom

occurs in laboratory crosses of ST/Y males. To test if the level of expression is approximately constant for a given 21/Y male, we crossed eight 21/Y males to ITA-24-P females and a month later crossed them again with another set of virgin females. The sexual proportions in their progenies (expressed as per cent males) were respectively for the two consecutive crosses and the eight males: 0.0, 1.2; 2.9, 2.5; 3.1, 1.1; 4.0, 3.0; 6.8, 6.4; 14.3, 15.0; 22.7, 10.6; 33.7, 27.4. The expression of the sex-ratio trait was highly correlated between the first and second cross ($r = 0.9398$, 6 df, $P < 0.001$) and the averages (10.94 ± 4.18 ; 8.40 ± 3.22 , respectively) are not significantly different.

DISCUSSION

The sex-ratio trait reported here in *D. mediopunctata* is clearly analogous to the one present in *D. obscura* (and other species): an X-linked factor, with sex-limited effect, which acts prezygotically in parental males, causing excess of females in progenies (Gershenson, 1928). Its association with chromosomal inversions is a feature found in all species investigated in this respect (Voelker, 1972).

One aspect of the *D. mediopunctata* case deserves special comment: the large variation in the effect of the *sex-ratio* factor, observable both in wild-caught (fig. 2) and laboratory reared (fig. 3) 21/Y males. Theoretical studies have demonstrated that the existence of an X-linked meiotic drive gene puts an "evolutionary prize" in autosomal and Y-linked modifiers (suppressors) of the drive (Eshel, 1975; Wu, 1983). Such modifier genes have been found in *D. paramelanica* (Stalker, 1961), in *D. affinis* (Voelker, 1972) but not in natural populations of *D. pseudoobscura*, despite specific search (Policansky and Dempsey, 1978; Beckenbach, Curtsinger and Policansky, 1982). The situation for *D. pseudoobscura* may change if the genetic systems described by Cobbs (1986, 1987) turn out to be relevant for natural populations.

If modifier genes are the cause of the variation in the expression of *sex-ratio* in *D. mediopunctata*, this species will be an interesting model to study the evolution of *sex-ratio* and other meiotic drive systems in natural populations.

We are presently investigating the nature of this variation. Our preliminary results strongly support the existence of autosomal and Y linked modifiers of the sex-ratio trait in *D. mediopunctata*.

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