A GENE THAT MODIFIES THE SEX RATIO IN A BISEXUAL STRAIN OF *SCIARA OCELLARIS*

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SUMMARY

The influence of an X-linked recessive mutation, *sepia*, on the sex determination of a bisexual strain of *Sciara ocellaris* was studied. It induces an alteration in the sex ratio, especially in the progeny of heterozygous females, increasing the proportion of males. These results cannot be explained by differential fecundity of the female parents of different genotypes nor by differential mortality between the sexes. The occurrence of gynandromorphs indicates that the mutant probably interferes with the processes of X-chromosome elimination. Observations that heterozygous females which received the *sepia* allele from their mothers produced a higher frequency of gynandromorphs than females which received the mutated allele from their male parents, suggest that the mutation is interfering with the mechanism of chromosome elimination present in the egg cytoplasm.

1. INTRODUCTION

SEX in Sciarid flies is determined by a selective chromosome elimination mechanism that is operative during the early nuclear divisions of the zygote (DuBois, 1932, 1933).

The sex ratio is also unusual because the females of some species produce progenies of a single sex, either males or females, and are known as "unisexual" species (Metz, 1931); others produce both males and females in their offspring, the "bisexual" species (Metz, 1938). Still other species regularly possess strains of both types, like *Sciara ocellaris* (Metz and Lawrence, 1938). In bisexual species the proportion of the two sexes in the progenies may vary widely rather than conforming to a 1 : 1 ratio (Metz, 1931, 1938).

Whether a unisexual female is a male-producer or a female-producer depends on the genetic constitution of the X-chromosome (Metz, 1931, 1938). No satisfactory explanation has been given, however, for the bisexual species and their variable sexual proportions. In the present report we describe the occurrence in a bisexual strain of *S. ocellaris*, of a mutation with pleiotropic effects, one of which is a modification in the sex ratio of the progeny.

2. MATERIALS AND METHODS

A laboratory bisexual strain of *Sciara ocellaris* (*Bradysia tritici*) Diptera, Sciaridae, was used throughout the work. The original wild type flies were captured in a greenhouse of the Biology Division, Oak Ridge National Laboratories, U.S.A., in 1966, and since then the strain has been maintained in the laboratory at the University of São Paulo, Brazil. The X-linked recessive mutation, *sepia*, appeared spontaneously in the stock. The most conspicuous effect of this mutation is a change in the dark grey, almost

black, parts of the wild type flies to a light brown colour. All the chitinous parts are affected except the eyes which remain black (Mori, Dessen and Perondini, 1973).

The homozygous wild type and *sepia* stocks were maintained in small cages according to the method of Rocha, Perondini and Magalhães (1975). Single pair matings were made in small vials containing a layer of 1 per cent plain agar and a constant amount of food. All experiments were done at 22°C.

Sciara ocellaris females lay all their eggs in about 4 days, after which they die. Egg counts were performed on the 5th day after mating and the counted eggs left to develop. The adults emerging from a known number of eggs in each cross will be referred to as adult survival. In experiments designed to test the sex-ratio in the progeny of specific pairs of matings, the same method was used as described above, except that egg counts were not made. The sex ratio is expressed as the relative proportion of males in a given progeny. Progenies with less than 30 flies were discarded.

The somatic tissue of the male flies is XO and will be represented by *s* or +; the female, which is XX, will be designated by two symbols, the first of which indicates the X-chromosome of maternal origin.

3. RESULTS AND DISCUSSION

The sex-ratio in the progeny of wild type crosses of a bisexual strain *Sciara ocellaris* is extremely variable, ranging from those producing very few males (1 per cent or less) to those with more than 95 per cent of males in the progeny (fig. 1). In the strain used in the present study, there is a

FIG. 1.—Frequency distributions of pair matings according to the relative number of males produced in their offspring. The number in each histogram indicates the type of cross as shown in table 1.

TABLE 1

Proportion of males in the offspring of several types of crosses and results of the Kolmogorov pairwise comparisons between the frequency distributions of crosses shown in fig. 1; the wild type crosses are taken as the standard

Crosses				Progeny		Kolmogorov		
Type	♀	♂	N	Mean % of males		Dn	D ₁ %	Obs
1	+/+	+	112	38.1 ± 2.62		—	—	—
2	s/s	s	94	50.4 ± 2.58		0.267	0.222	**
3	+/s	s	62	74.0 ± 2.52		0.530	0.257	**
4	+/s	+	116	70.8 ± 2.84		0.465	0.218	**
5	s/+	+	117	65.0 ± 2.36		0.417	0.221	**

** Significant.

predominance of pair matings producing less than 50 per cent of males in the offspring (fig. 1, table 1).

The proportion of males in progenies was higher when the mutant *sepia* was introduced into the crosses (fig. 1). Taking the distribution of the wild type crosses as standard, the Kolmogorov statistics showed (table 1) that all the distributions were statistically different from the wild type one.

The fecundities of the females with various genotypes are shown in table 2. An analysis of variance of these data showed that differences in egg laying could not be the cause of the modification observed in the sex ratio ($F_{7,79} = 1.069$, n.s.).

The data on relative adult survival was normalised by *arc-sin* transformation and the values compared by an analyses of variance, resulting in a significant value ($F_{7,79} = 11.55$). The Scheffé test showed that only the relative adult survival of homogamic crosses for *sepia* was statistically different from the rate of survival observed for the wild type matings.

A higher mortality of *s/s* females could explain the increased relative proportion of males in the progenies of crosses homogamic for *sepia*. This hypothesis cannot be tested directly, since no expected value for the proportion of males in a given progeny exists in this bisexual strain of *S. ocellaris*. In the crosses involving heterozygous females differential mortality favouring the males cannot explain the observed deviation in the sex ratio, as shown in table 1.

An insight into the mechanism that may be involved in the modifications of the sex ratio by *sepia* was obtained from an analysis of mosaic flies that

TABLE 2

Fecundity and adult survival

Crosses				Fecundity*	Mean % of adult survival
Type	♀	♂	N		
1	+/+	+	10	165.8 ± 11.22	85.1 ± 5.19
2	s/s	s	14	161.7 ± 5.89	51.1 ± 2.26**
3	+/s	s	12	161.6 ± 14.45	69.2 ± 3.33
4	+/s	+	9	164.3 ± 14.02	78.7 ± 5.66
5	s/+	+	12	187.0 ± 16.11	76.1 ± 2.97

* Mean number of eggs per female ($\pm$ s.e.).

** Statistically different from survival of wild type cross.

appeared during the course of the studies. The chromosomal constitution of these mosaics was inferred from an analysis of a proportion of them in which the mosaicism affected anatomical parts that exhibited a sexual dimorphism, such as the wings and the external genitalia. In the mosaic flies, produced by heterozygous females crossed to $+$ males, the mosaic genitalia always exhibited the *sepia* colour on its male structures, and hence, must have had an XO constitution. In contrast the female parts were of the wild type and, probably, had a heterozygous genotype. When the mosaicism affected the pair of wings, the *sepia* one was smaller than the wild type on the other side. Since *Sciara* males, in any given strain, are smaller than the females (Rasch, 1970), we concluded that the *sepia* wing had male tissues (XO) and the larger wild type ones, female tissues (X^sX^+).

In crosses of heterozygous females with s males two types of mosaics were observed. One showed a mixture of *sepia* and wild phenotype, as described above except that the X^s -chromosomes in the XO parts were derived from the male parent. The other type of mosaic showed a homogeneous wild type phenotype, but had one wing smaller than the other and a mixture of male and female structures in their external genitalia. These flies were probably composed of X^+O and X^+X^s tissues, the X^+ -chromosomes in the XO parts being a maternally derived chromosome. On the basis of these observations we conclude that the mosaics we have described are gynandromorphs exhibiting a variable mixture of male and female parts.

According to previous authors (DuBois, 1932, 1933; Metz and Lawrence, 1938; Metz, 1942, 1959; Crouse, 1943; Crouse *et al.* 1971) the gynandromorphs observed in sciarid flies are a consequence of errors in the elimination of X-chromosomes in early embryogenesis. The same interpretation may explain the origin of the gynandromorphs observed in the present analysis. Since no mosaicism for the wings and for the external genitalia was observed in the progenies of homogamic crosses of the wild type (11,673 individuals) or of *sepia* flies (7,370 individuals) we assume that the *sepia* mutation was interfering with the processes of chromosome elimination in the embryo and producing the gynandromorphs and that this interference occurs only in heterozygotes.

The system of X-chromosome elimination in *Sciara* is based, apparently, on two additive mechanisms. During male meiosis the chromosomes receive an imprint which permits them to be recognised in the embryo as paternally derived chromosomes (Crouse, 1960, 1966, 1977). In the cytoplasm of the zygote there must exist a second mechanism for the recognition of these imprinted chromosomes and which causes their elimination during the fifth to ninth cleavages of the zygote nuclei (DuBois, 1933; Crouse, 1960; Rieffel and Crouse, 1966).

The *sepia* mutation could exert its effects by modifying one, or both, of these mechanisms. It was observed that heterozygous females which received the *sepia* allele from their mothers ($s/+$) produced 1.38 and 1.66 per cent of gynandromorphs when crossed to $+$ and s males respectively. However, the frequency of these aberrant flies was lower in the progenies of $+/s$ females. When crossed to $+$ and s males these females produced 0.37 and 0.66 per cent respectively of gynandromorphs in their progenies. These facts suggest that *sepia* exerts a maternal effect on the mechanism of chromosome elimination present in the egg cytoplasm causing errors in the elimination of the X-chromosome and the production of gynandromorphs. This

interference seems not to act directly on the chromosome because in the different types of gynandromorphs described here, either X^s or X^+ could be eliminated. Another fact that reinforces this conclusion is the observed 1:1 ratio of + and s males in the progeny of heterozygous females (data not shown). This putative effect of *sepia* would also explain the observed modification in the sex ratio, especially in the progenies of heterozygous females. Under the influence of *sepia* two X-chromosomes would preferentially be eliminated resulting in a larger proportion of male individuals.

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