

# INHERITANCE OF THE X CHROMOSOME IN EXCEPTIONAL MALES OF *SCIARA OCELLARIS* (DIPTERA)

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## INTRODUCTION

THE present paper represents an attempt to throw further light on the subject of sex determination and the sex chromosome mechanism in *Sciara*. Earlier work in this field has been reviewed by METZ (1938), and a brief review here will suffice. In some species of *Sciara* and in some strains of other species, females produce progenies, or families, which are all or nearly all of one sex. In such a progeny the few individuals of the other sex which may occur are called exceptional males (in female progenies) or exceptional females (in male progenies). It was found by METZ that males normally get their somatic X chromosome from the female parent. CROUSE and SMITH-STOCKING (1938) state that there are many cases of irregularity in the inheritance of the X chromosome in males when the yellow mutant strain of *S. ocellaris* is crossed with wild type. The irregularity does not appear to be causally associated with the mutant gene. A sex-linked mutant gene is necessary as a marker in order to detect the irregularity, which is presumably occurring also when a marker is not present. The evidence given below indicates that the inheritance of the X chromosome is irregular only in exceptional males and, to some extent, in males of "bisexual" progenies which are predominantly female, and further that exceptional males which retain the paternal X chromosome are sterile.

In *S. coprophila* female-producing females differ from male-producing females by the possession of a modified X chromosome, designated X' (X prime). Female producers are heterozygous (X'X), while male producers are homozygous for the normal X chromosome (XX). All females which receive the X' chromosome are female producers. The two types of females are apparently produced in a 1:1 ratio. In *S. ocellaris*, on the other hand, the ratio of males to females within a progeny varies greatly. Some strains behave much like *S. coprophila* and give "unisexual" progenies. Others give both sexes in varying proportions. It has been found in studies as yet unpublished that some females from a "bisexual" strain may give "unisexual" progenies while their sisters yield progenies of various proportions of males and females all the way to a ratio of 1:1. Generally in such cases the "unisexual" progenies of sisters are of the same sex and the "bisexual" progenies of their sisters tend to have more individuals of this sex than of the other sex. In other cases the progenies of sisters may be predominantly about the 1:1 ratio, or the mode may apparently be around any arbitrary ratio, or the distribution may be bi-modal. In both "unisexual" and "bisexual" strains of *S. ocellaris* male producers and female producers frequently are not produced in a 1:1 ratio. Tests for the X' chromosome in *S. ocellaris* show that it does not exist sharply distinguishable

from the X chromosome as in *S. coprophila*. Although the studies are not yet complete, they indicate that X chromosomes have various tendencies and potencies in the X' direction and that factors which influence sex are present also on the autosomes.

In the oögenesis of *Sciara* the maturation divisions show no unusual characteristics, but in spermatogenesis meiosis is quite atypical. Two X chromosomes occur in each sperm. These chromosomes are genetically equal, the products of the division of a single X chromosome which went precociously to one pole of the spindle in the second meiotic division. The chromosomes which go to the other pole are budded off. Thus the fertilized egg contains three X chromosomes, of which one or two are eliminated from the somatic tissue in early cleavages, depending on whether the individual is to become a female or male. Normally one of the two X chromosomes from the sperm is eliminated in individuals to become females, while in those to become males both X chromosomes contributed by the sperm are eliminated, leaving only the one of maternal origin.

Elimination of a chromosome also takes place in the germ line of the developing embryo, but this differs from that in the soma. One X chromosome is normally eliminated in both males and females, and this chromosome is ordinarily one of the two paternal X chromosomes. Thus the spermatogonia and oögonia each have two X chromosomes, one of maternal and the other of paternal derivation. Males transmit only the one of maternal origin.

In "unisexual" strains the cytoplasm of the eggs appears to be conditioned by the genetic constitution of the mother so as to pre-determine to some extent the nature of chromosome elimination from the soma during development. Thus the eggs of male-producing females show a tendency to eliminate the two paternal X chromosomes during development, yielding males. In the female-producing females the tendency is to eliminate only one X chromosome from the soma. An exceptional male is produced by an egg which would be expected to eliminate only one X chromosome from the soma but which actually eliminated two.

Irregularities in chromosome elimination from the soma can be detected only when the zygote is heterozygous for a sex linked factor. There are several ways in which such irregularities could occur in males. Instead of the selective elimination of paternal X chromosomes just mentioned, selection might be at random among the three X chromosomes. If the female parent is homozygous and different from the male parent, this would result in a 2:1 ratio of paternal and maternal phenotypes, respectively. This may be more easily visualized by illustrating it as follows. (Since the two X chromosomes contributed by the male are the product of the division of one chromosome during spermatogenesis, only one is represented in the male.)  $y/y \text{ ♀} \times Y \text{ ♂} \rightarrow yYY \text{ zygotes} \rightarrow 2Y:1y$  among the phenotypes of the male offspring. If the female parent is heterozygous the ratio of paternal to maternal phenotypes would be 5:1.  $Y/y \text{ ♀} \times Y \text{ ♂} \rightarrow YYY + yYY \text{ zygotes} \rightarrow 5Y:1y$ , or  $Y/y \text{ ♀} \times y \text{ ♂} \rightarrow Yyy + yyy \text{ zygotes} \rightarrow 5y:1Y$ .

If one X chromosome contributed by the male were eliminated as usual and selection were at random between the other paternal and the maternal X

chromosome, the ratio of phenotypes among the male offspring would be 1:1 when the female is homozygous and different from the male, and 3:1 when the female is heterozygous. This may perhaps be best represented by putting in parenthesis the symbol for one of the paternal X chromosomes, leaving random selection between the other two.  $y/y \text{♀} \times Y \text{♂} \rightarrow yY(Y)$  zygotes  $\rightarrow 1y:1Y$  among the phenotypes of the male offspring.  $Y/y \text{♀} \times Y \text{♂} \rightarrow YY(Y) + yY(Y)$  zygotes  $\rightarrow 3Y:1y$ , or  $Y/y \text{♀} \times y \text{♂} \rightarrow Yy(y) + yy(y)$  zygotes  $\rightarrow 3y:1Y$ . Other ratios would occur if there were selection favoring the retention of an X chromosome from one parent or the other.

EXPERIMENTAL DATA

Table 1 shows the ratios expected on the basis of the kinds of selection mentioned and gives the values observed. The two mutant genes concerned

TABLE 1

*Ratios expected on the basis of four types of selection of the somatic X chromosome of male offspring, and the values observed. The types of selection listed are: I. Random selection among all three X chromosomes present in the fertilized egg. II. Random selection between the X chromosome of maternal origin and one of the X chromosomes of paternal origin. III. Selection of the maternal X chromosome. IV. Selection of a paternal X chromosome.*

Genotypes of parents	♀ $y/y \times Y$	♂ $Y/Y \times y$	♀ $Y/y \times Y$	♂ $Y/y \times y$	♀ $yC/yC \times yC$	♂ $YC/yC \times yC$
Phenotypes of offspring	Y:y	Y:y	Y:y	Y:y	yC:yC	YC:yC:yC
Expected ratios						
I	2:1	1:2	5:1	1:5	1:5	1:1:4
II	1:1	1:1	3:1	1:3	1:3	1:1:2
III	0:1	1:0	1:1	1:1	1:1	1:1:0
IV	1:0	0:1	1:0	0:1	0:1	0:0:1
Observed values	F 23, 1 G 35, 2	A 29, 31	A 29, 34 F 11, 0	F 3, 45 G 5, 29	Ja 6, 12 Jb 35, 36	Ja 6, 3, 3 Jb 59, 55, 4

are sex-linked recessives, yellow (body) and curled (wing). Since exceptional males are few in number, the values represent males from several groups, each group designated by a letter. The female parents yielding the flies in each group except A were sisters. In group A the males represent several generations of closely related flies.

An observed ratio may be compared with an expected ratio by the method of the binomial expansion or the method of chi-square, both too familiar to warrant description. An observed ratio is said to be significantly different from an expected ratio when the probability of obtaining it or a ratio farther from the expected is not greater than one chance in twenty. The difference between them is said to be highly significant when the probability is not greater than one chance in a hundred. The method of the binomial expansion is the more accurate. The inaccuracy of the chi-square method is considerably greater when the numbers involved are small, and in such cases the binomial expansion should be employed. When the numbers are large the chi-square method is

usually sufficient to ascertain significance, and the binomial expansion need be used only as a check in borderline cases.

In group A in table 1, the exceptional males from the cross  $Y/Y$  by  $y$  fit very well the 1:1 ratio (29 $Y$ , 31 $y$ ). They are significantly and nearly highly significantly different from the 1:2 ratio. It seems therefore that they indicate the elimination of one of the paternal X chromosomes from the fertilized egg and random selection between the other two. In the cross  $Y/y$  by  $Y$  for the same group the ratio is again nearly 1:1 (29 $Y$ , 34 $y$ ), but here it indicates a strong selection for the X chromosome of maternal origin. Its difference from both the 5:1 and 3:1 ratios is highly significant. The reason for the lack of uniformity in the results of the two kinds of crosses in this group is not known.

In group F there seems to be a strong selection favoring an X chromosome of paternal origin in the cross  $y/y$  by  $Y$ . The numbers (23 $Y$ , 1 $y$ ) are highly significant in their difference from both the 2:1 and 1:1 ratios. In the other two crosses ( $Y/y$  by  $Y$ , 11 $Y$ , 0 $y$ ; and  $Y/y$  by  $y$ , 3 $Y$ , 45 $y$ ) the trend is also strongly in this direction. The first is not significantly different from the 5:1 ratio expected on the basis of random assortment of all three chromosomes. The second differs significantly from the 1:5 ratio (by the method of the binomial expansion, though not by chi-square). The first differs significantly from 3:1 (method of binomial expansion) and highly so from 1:1. The second differs highly significantly from both 1:3 and 1:1. The tendency in this group as a whole appears to be toward strong selection of a paternal X chromosome.

In group G the trend is similar to that in group F. In the cross  $y/y$  by  $Y$  (35 $Y$ , 2 $y$ ) there is no question of it, while in the cross  $Y/y$  by  $y$  (5 $Y$ , 29 $y$ ) all the ratios are probable except 1:1, which is highly improbable. Groups F and G are very closely related, since the female parents of the two sets of sisters which produced these progenies were sisters and the male parents were brothers.

In group J the progenies which were predominantly female were essentially "bisexual," while those which were predominantly male were "unisexual." An arbitrary limit was drawn to separate the "unisexual" from the "bisexual" progenies. The males of those in which there were less than ten males per hundred females are listed in table 1 as Ja, while the males of those in which there were ten or more males per hundred females are listed as Jb. This seems to be a fairly satisfactory distribution. In "unisexual" strains there are generally not more than three or four exceptional males in a progeny, when any, but the progenies are usually less than a hundred. In the first cross ( $yC/yC$  by  $yc$ ) there is in Jb a 1:1 ratio (35 $yC$ , 36 $yc$ ), indicating that the X chromosome in all or nearly all males came from the female parent. The difference between these values and the 1:3 ratio expected if selection is at random between a paternal and the maternal X chromosome is highly significant. In Ja the numbers are 6 $yC$  and 12 $yc$ . Here there may be paternal X chromosomes altering the ratio, but the numbers are too small to be significant. In the second cross ( $YC/yC$  by  $yc$ ) one can tell just where each X chromosome came from. In Jb four out of 118 males got the paternal X chromosome and in Ja three out of twelve. When these numbers are set in the two-by-two table it is found that

the difference between Ja and Jb is highly significant, since chi-square is 9.98. Thus Ja and Jb do not represent samples from the same population with regard to the number of males which received the paternal X chromosome. In those considered to be from "unisexual" progenies a significantly larger proportion got it than in those of the "bisexual" progenies. The males of male progenies in group J all received the maternal X chromosome. The males in predominantly female "bisexual" progenies are intermediate between exceptional males and normal males in that their proportion in the progeny is greater than that of exceptional males but not so great as that of normal males, which occur alone or with but a few females. It seems that where there is a tendency for exceptional males to inherit the paternal X chromosome; this tendency is less marked in closely related males of predominantly female "bisexual" progenies.

In males of male progenies the inheritance of the X chromosome has been regularly from the female parent. In more than five thousand cases from the crosses  $Y/Y$  by  $y$  and  $y/y$  by  $Y$  only one exception has been found, and this may have been an error.

Thus normal males consistently inherit the maternal X chromosome, while in the case of exceptional males there seems to be in different groups of closely related males various tendencies to inherit one of the paternal X chromosomes. The evidence at hand further indicates that in males of "bisexual" female progenies the tendency to inherit a paternal X chromosome is not so great as in closely related exceptional males.

"Bisexual" and "unisexual" strains of *S. ocellaris* are interfertile. As has been mentioned, a great variety of ratios of males to females may be obtained in different progenies. It may vary from one extreme to the other—from all males to equal numbers of males and females to all females. In predominantly female "bisexual" progenies the ratio may be significantly different from 1:1 and still contain a considerable number of males. It appears that in such cases the predisposition toward femaleness is not so great as in progenies which have only a few exceptional males. Irregularities in the inheritance of the X chromosome are common among exceptional males but do not appear to occur among normal males. These irregularities seem to be associated with something which causes the elimination of two X chromosomes instead of one from the soma in the presence of a predisposition toward femaleness (toward the elimination of one instead of two), but not associated with it in the absence of this predisposition toward femaleness. When the predisposition toward femaleness is less—that is, in "bisexual" progenies—the number of irregularities might, on this basis, be expected to be smaller. The evidence from group J indicates that this is so.

Twenty-four exceptional males which had received their X chromosome from the male parent were mated, many of them with six females each. All were sterile though they mated readily. An unrecorded number of these were dissected, and all had rudimentary gonads with no sperms in them or in their ducts. Several hundred similar dissections were made on normal males, and though variation was found, the gonads generally were large and they and

their ducts were filled with sperms. The rudimentary gonads of the sterile males were lumpy in form. Some of the gonads of normal males had at their distal ends masses of clear, lumpy material similar to the gonads of the sterile males. Gonads were removed from an unrecorded number of exceptional males which got their X chromosome from the female parent. All were found to be smaller than the average size of those from normal males. One such pair of gonads which were removed after the male had mated were about the size and condition of those from a sterile male of the paternal X chromosome type, but there were sperms in the duct. This male proved to be fertile. Others were somewhat larger and contained sperms but with much of their bulk made up of the clear, lumpy material characteristic of the sterile ones.

From these observations it appears that the sterility is caused by the lack of sperms and not because of abnormal chromosome numbers in the sperms or failure of the males to mate. It is not known what kind of chromosome elimination takes place in the germ line of these males. Since it is abnormal in the soma it may be abnormal in the germ line also. Normally in both males and females one paternal X chromosome is eliminated from the germ line during development, leaving the other paternal one and the maternal one. In the first meiotic division of spermatogenesis a mono-polar spindle is formed, and all the chromosomes of maternal origin go to the pole, and those of paternal origin go away from it and are budded off. If the maternal X chromosome is eliminated from the germ line as well as from the soma and at the first meiotic division the paternal chromosomes go away from the pole and are lost, there would be no X chromosome left, and spermatogenesis might be unable to continue without one. This, however, would not explain the condition in exceptional males which got their somatic X chromosome from the female parent and which have small abnormal gonads but are fertile. Exceptional males may be considered males which should have been females. The factor which is in the eggs of a female-producing female which tends toward the elimination of only one X chromosome instead of two—a tendency toward femaleness—may persist in the exceptional males and interfere with spermatogenesis. It would be necessary to assume further that this environment is less compatible to an X chromosome from the male parent than to a maternal one. It would be well to study the gonads of males from predominantly female "bisexual" progenies to see if they are intermediate between those of normal and of exceptional males and to see if a correlation exists between the size of the lumpy mass on fertile gonads and the sex ratio of the progeny. This work has been interrupted by the war.

#### SUMMARY

Males of male progenies regularly inherit the maternal X chromosome, only one possible exception being observed in more than five thousand cases. Among exceptional males there may be a strong tendency toward the inheritance of the maternal or of a paternal X chromosome or there may be a random selection between the maternal and a paternal X chromosome. In the cases observed there was consistency in the type of selection within each group made

up of the offspring of sibs. There is evidence that in males of predominantly female "bisexual" progenies the amount of inheritance of a paternal X chromosome is less than that in closely related exceptional males. Exceptional males which inherit an X chromosome from the male parent are sterile.

## LITERATURE CITED

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