GENETIC IDENTIFICATION OF THE SEX CHROMOSOMES IN SCIARA (DIPTERA)[1]

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Communicated January 10, 1929

The peculiar relationship between the chromosomes of the two sexes in Sciara[2] and the peculiar chromosome behavior during spermatogenesis,[3] together with the regular production of "unisexual" progenies, due to the presence of male-producing and female-producing females,[4] have presented a series of phenomena difficult to harmonize on any simple scheme of sex determination. Perhaps the greatest difficulty in this respect is presented by the two large "sex limited" chromosomes, found only in males,[5] but apparently transmitted by all sperms. These chromosomes differ from the others in size and shape and remain condensed during the growth period of the primary spermatocyte in the manner typical of sex chromosomes. Thus their form and cytological behavior would indicate that they are sex chromosomes, but their distribution is such as to make it difficult to outline any simple scheme of sex determination based on the assumption that they are the primary sex determiners.

Without attempting a detailed discussion of the question of sex determination at this time, we present the accompanying summary of data which, we believe, demonstrate the presence of a pair of sex chromosomes distinct from the "sex limited" chromosomes just mentioned. These sex chromosomes are present in both males and females—the males being XY and the females XX in constitution.

The evidence is based on the inheritance of "swollen" wing veins, a clear-cut recessive mutant character. This character has been followed through more than ten generations and has shown typical sex-linked inheritance, except in one experiment discussed below and here the disturbance appears to be superimposed on the regular type of inheritance.

Swollen was first observed (by Ullian) in four males, the only offspring from a mass mating. These males were mated to wild-type virgin females from a related culture. From this mass mating 9 wild-type females and 7 wild-type males were obtained. The females were divided into three lots, which were outcrossed, two to males from wild-type stock and one to males from a related culture. The three matings gave the following results, in the order named:

(1) 84 wild-type females, 14 wild-type males and 10 swollen males;
(2) 144 wild-type females, 2 wild-type males and 1 swollen male;
(3) 55 wild-type females, 27 wild-type males and 34 swollen males.

In interpreting these results it must be remembered that individual females give "unisexual" progenies[5] and that the matings are mass matings. The ratio of females to males, therefore, is of no significance. Making allowance for this fact, the results are those expected on the basis of ordinary sex-linked inheritance—i.e., half the sons but none of the daughters are swollen.

Later matings involving heterozygous females by swollen males gave wild-type and swollen flies of both sexes as expected. But during this period and for some time afterward poor conditions in the cultures made the counts very small and unsatisfactory. Furthermore, the stocks used at that time were so interrelated that none was known definitely to be free from swollen. Consequently we may omit the records from these experiments (although they consistently indicate sex linkage) and consider only the subsequent ones in which such difficulties were eliminated.

In the latter experiments virgin swollen females were outcrossed singly to males from a wild stock just secured in California.[6] From such matings the sons were all swollen and the daughters all wild-type, as expected.

Since in this material "unisexual" progenies are obtained, the males and females come mainly from different parents. Seven mothers gave 212 wild-type daughters and seven others gave 355 swollen sons and 9 wild-type "exceptional" daughters. One bilateral gynandromorph appeared in which the male side was swollen and the female side wild-type.

Twenty-five F_1 females from matings of this kind were then tested individually by mating to swollen males from the same cross. Ten of these were "female-producers" and gave a total of 526 wild-type females, 1 swollen female, 7 wild-type males and 6 swollen males. The remaining fifteen were "male producers" and each gave both wild-type and swollen sons—total 261 wild-type and 369[7] swollen sons and one wild-type "exceptional" daughter.

At the same time five of the F_1 females were mated to wild-type males. One gave only wild-type daughters; the other four all gave only sons, both wild-type and swollen.

In the above tests of F_1 females the male progenies all give the results expected on the basis of ordinary sex-linked inheritance—i.e., the offspring are approximately half swollen and half wild-type. But in the female progenies, where the mothers were mated to swollen males and therefore should give both types of offspring, the expected swollen class is absent. Only one such female appeared where approximately 500 would be expected. This is the exceptional behavior mentioned above. That the mothers here were all actually heterozygous for swollen is made practically certain by two facts: first, that their male-producing sisters showed such a constitution and second, that six of the ten female-producers themselves gave "exceptional" swollen sons.

It is possible that the absence of swollen females in this case is due to poor viability, for such females have proved to be consistently less viable than the wild-type throughout our experiments. However, no such extreme inviability as this has been shown in any other experiment. Another possibility is that genetically swollen females were present here in the expected proportion, but failed to manifest the character somatically. This explanation is practically ruled out, however, by the fact that in other experiments no such behavior has been indicated and also by genetic tests of the females in question, as shown below.

Twenty-six of these females were tested individually by mating to swollen males. Fifteen were female-producers, all of which gave both wild-type and swollen daughters (total 433 wild-type, 218 swollen) thus showing that they were heterozygous. The remaining eleven, which were male-producers, gave similar results (total 389 wild-type, 349 swollen) except in one case. In the latter the eighteen offspring were all swollen. The mother may, therefore, have been homozygous for swollen in this case, although the presence of a sex-linked lethal might account for the result equally well.

In succeeding generations from this same series the inheritance has been that of a typical sex-linked character. For instance, in the generation following that just considered, twenty heterozygous female-producing females were tested and all gave both wild-type and swollen daughters. In four of these cases the actual numbers were not recorded. The other sixteen gave a total of 259 wild-type females, 120 swollen females, 4 wild-type males and 8 swollen males. In the next generation forty-four heterozygous female-producers were tested, with similar results, except in one case where only sixteen offspring were secured. The heterozygous male-producing females in these experiments have consistently given both wild-type and swollen sons, as expected, regardless of the type of males to which they were mated. Likewise, from outcrosses of swollen females to wild-type males the daughters have regularly been all wild-type and the sons all swollen, as expected, and from outcrosses of swollen males to wild-type females the offspring have all been wild-type as expected.

Reviewing the evidence as a whole, therefore, we see that all the possible types of matings have been made and all have given results indicating typical sex-linked inheritance, except in the one generation following the outcross to California stock as discussed above. The absence of swollen females in this generation is presumably due to some genetic difference between the two stocks used, but in any case it appears to bear no relation to the problem under consideration and may be left out of account here.[8]

Since the genetic evidence reveals the type of inheritance expected where the female is XX and the male XY we conclude that sex chromosomes of this type are present[9] and are responsible for sex determination.

Such a conclusion leaves the rôle of the "sex-limited" chromosomes unexplained and also introduces difficulties in any explanation of the "unisexual" progenies, for which the female is responsible. These features will be considered subsequently.

Summary.—See first two and last paragraphs.

[1] This investigation has been aided by a grant from the NATIONAL RESEARCH COUNCIL, Committee for Research on Problems of Sex.

[2] Metz, C. W., *Amer. Nat.*, **40**, 42–56, 1926.

[3] Metz, Moses and Hoppe, *Zeits. ind. Abs. Vererb.*, **42**, 237–270, 1926.

[4] Metz and Moses, *Anat. Rec.*, **34**, 170, 1926. Moses and Metz, these PROCEEDINGS, **14**, 928 (Dec., 1928.)

[5] These have no counterparts in the females, which thus have two chromosomes less than the males.

[6] All our previous material had been secured in the eastern states.

[7] In the males the proportion of swollen flies often exceeds 50%. The reason for this is not yet clear, but in any case it apparently has no bearing on the main question under consideration.

[8] This particular type of cross is being repeated to see if the result is due to a difference between the two strains of flies used. Such a difference might, e.g., result in a selective segregation of chromosomes in the female-producing female analogous to that which has been found regularly in the male. Cf. Metz, C. W., *Zeits. ind. Abs. Vererb.*, **45**, 184–201, 1927.

[9] The presence of Y is inferred from cytological evidence; see papers referred to in footnotes 2 and 3 above.

A SPONTANEOUS MUTATION IN THE HOUSE MOUSE

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Communicated December 26, 1928

In the 9th brother-sister generation of an inbred line of chinchilla non-agouti (black) piebald mice a single female, No. 7048, was discovered, all of whose ventral hairs (with the exception of a small black area between the forelegs) were white. This ventral lightness was not an extension of piebald spotting, the white being less intense than piebald white, so that a small belly spot of lighter hue due to the piebald factor was quite distinct.

That this animal represented a mutation was apparent for the following reasons: (1) Its parents ♀ 6383 and ♂ 6382 produced in all 14 young in four litters, only one of which, No. 7048, showed the ventral coloration described; (2) ♀ 7048 bred to totally unrelated non-agouti dark-bellied males produced about equal numbers of white-bellied to dark-bellied young (20 light-bellied black: 10 dark-bellied black); (3) three of her sis-