INTRODUCTION

THE unusual behavior of the chromosomes in Sciara has been studied in considerable detail both cytologically and genetically. Although the main facts regarding the distribution and behavior of the chromosomes (as revealed by cytological examination), have been known for some time, the genetic data have been incomplete because of the paucity of mutant characters with which to follow the history of the individual chromosomes in any one species. The present paper is designed to supply additional genetic data derived from an intensive study of *Sciara coprophila* Lintner, over a period of five years. Because of the great difficulty of securing mutant characters in Sciara, only a few have been obtained, even with the aid of X-rays, and some of these are unsatisfactory. Even the latter have been included, however, in order to make the account as complete as possible. The main results of this study were presented by Smith (1932) but publication of the present account has been delayed in the hope of securing more and better characters—a hope that has not been realized.

Previous genetic studies on this species have dealt with one pair of autosomes (Metz 1927, truncate wings), and the sex chromosomes (Metz, Ullian, Schmuck, Smith 1929–31). The new characters considered here make it possible to present the essential facts concerning the behavior of the remaining pairs of autosomes, as well as additional data concerning the first pair.

Before analyzing the genetic results in detail, it is necessary to review briefly certain aspects of chromosome behavior in Sciara. One feature of importance is the difference in chromosome number between the two sexes, and between the soma and germ line in each sex (Metz 1931). These differences are brought about by a process of chromosome elimination during cleavage in the developing egg (Dubois 1932). The typical number of chromosomes in somatic groups is eight in the female (one pair of V’s, three pairs of rods); and seven in the male (one pair of V’s, two pairs of rods, one single rod). These are shown in figure 1a and 1b. The male group is similar to the female group but lacks one rod, presumably a sex chromosome. There are present in the germ-line of both sexes, one, two, or three
additional chromosomes which are longer and thicker than the ordinary chromosomes. These are termed the “limited” chromosomes (fig. 1c), (Metz 1931, Metz and Schmuck 1931). Since they are found only in the germ-line, they cannot be studied by means of somatic characters and consequently will not be considered in this account. The evidence indicates, however, that these chromosomes are relatively empty of genes (Metz 1929), and that they are not true sex chromosomes.

Another phenomenon of special significance in the present study is the occurrence of a “monocentric” mitosis at the first spermatocyte division (Metz 1926, Metz, Moses and Hoppe 1926, Metz 1933). This mitosis is accompanied by a selective segregation of chromosomes. During prophase at this division, the chromosomes are distributed at random throughout the nucleus. Although the chromosomes are present in pairs, no evidence of synapsis has been observed at any stage of spermatogenesis. A half spindle is formed with a single pole to which all the chromosomes are attached by “spindle fibres.” Subsequently, without forming an equatorial plate, they move directly into anaphase. Both of the “limited” chromosomes go regularly toward the pole, but the others segregate in such a way that one member of each pair goes toward the visible pole and the other away from it, despite the fact that the “spindle fibres” of all extend toward the pole.

When the four retreating chromosomes reach the periphery of the cell, they are deflected in their course, as if the cell wall were a mechanical barrier, and eventually they come together in a group opposite the pole. Later they are extruded in a bud resembling a polar body, and take no further part in development. From the cytological evidence, it is clear that the chromosomes are distributed here in a definite and regular way, so that one group is left in the functional cell, and the other is discarded. The question now arises as to which chromosomes are retained and which are cast off, and what is the nature of the forces operating to produce this result.

The first evidence bearing on the problem was obtained through a study of the character “truncate wings” in Sciara coprophila (Metz 1927). Truncate is a recessive autosomal character, somewhat similar in appearance
to truncate in *Drosophila melanogaster*. It was found that the gene for truncate was transmitted through the females in the ordinary Mendelian fashion with random segregation. The males, on the other hand, bred as though they were homozygous for the gene received from their mothers, and did not transmit the gene received from their fathers. This fact indicated that in the case of one autosome pair segregation was selective, and that the autosome retained in the functional cell at the first spermatocyte division was regularly maternal in origin. It remained to be determined how far this behavior was characteristic of the other chromosomes of this species.

A second chromosome pair was identified when two recessive characters, swollen and narrow, (Metz and Ullian 1929, Metz and Schmuck 1931) were found to show typical sex-linked inheritance. Later a dominant character, Wavy, (Metz and Smith 1931) was secured, and more recently, two additional recessive characters, round and miniature, (Smith-Stocking, unpublished) appeared which show the same type of sex-linked inheritance. The first evidence suggested that the sex chromosome complex of the female was XX and that of the male was XY. Later however the male soma was found to contain only seven chromosomes, indicating that it has no Y chromosome. The male germ-line possesses two sex chromosomes. One of these is evidently the same as the X in the somatic cells, but the precise nature of the other is obscured by a series of phenomena which are not yet fully analyzed and which need not be reviewed here (Metz 1934). The evidence is consistent, however, with the view that these two undergo the same type of selective segregation as that shown by the autosome pair just considered.

In each of two other species of Sciara a character was found which was inherited in the same way as truncate wings, suggesting that perhaps this unusual chromosome segregation is typical of the genus (Metz 1928, Metz 1929).

The present genetic study was undertaken for the purpose of analyzing the method of segregation of all the chromosomes of one species. It has involved: (1) the securing of new mutant characters, sufficient in number to identify each pair of chromosomes, and (2) analyzing the relationship of the new characters to each other by means of linkage tests to ascertain whether all of the chromosomes observed cytologically (save the “limited” chromosomes) were accounted for genetically. *Sciara coprophila* was used since it is the most satisfactory species for laboratory purposes. Although the sex chromosomes and one pair of autosomes had been previously identified genetically, there remained two pairs of autosomes to be studied.

Because of the difficulty of securing mutant characters, it has been necessary in the present study to make use of some characters which are
inconstant and otherwise unsatisfactory. This has not only increased the
task of making genetic tests, but has also necessitated presenting here a
more detailed description of the experiments than would otherwise be re-
quired. An account of culture methods and breeding technique is included,
since it has not been fully treated in earlier papers.

CULTURE METHODS AND BREEDING TECHNIQUE

1. Culture medium

Sciara is cultured in glass vials one inch wide and four inches deep. These are sterilized and filled to a depth of approximately one inch with
an agar solution made by heating together equal parts of agar-agar and
water. To insure a dry surface, a small amount of sterilized ground straw
is sprinkled into the vials after the medium has solidified. The vials are
plugged with cotton. Half pint milk bottles may be used for maintaining
mass cultures.

2. Life history and food

The genus Sciara belongs to the group of so-called fungus gnats, some
species of which inhabit mushroom beds and often become a serious men-
ace to commercial enterprise. The adult of S. coprophila is small, dark, and
inconspicuous.

The flies are usually cultivated in pair matings, a single female and one
or more males being placed in each vial. Since it has been shown that a
given female produces offspring from only one male (Moses and Metz
1928), several males are often placed with one female as a precaution
against possible sterility. Copulation usually takes place soon after the
flies are placed together. The sperms are stored by the female in the
spermathecae and the eggs are fertilized one by one as they leave the
vagina. They are deposited on the surface of the agar and hatch into small
transparent larvae in about six days, at which time they must be fed.

Many types of food have been tried with varying degrees of success.
The most satisfactory one found thus far is a mixture consisting of equal
parts of animal-poultry yeast, powdered mushroom, and straw. The latter
serves to prevent the formation of an impervious surface layer on the cul-
tures. When small larvae are visible (usually ten days after the parent
flies have been placed in the vial), a small quantity of food mixture is
sprinkled on the surface. This is soon eaten and the supply must be re-
plenished about every second day until pupation begins. Practice alone
will demonstrate what quantity of food is required. In general it is better
to feed sparingly rather than abundantly, for if cultures are given too much
food, the larvae fail to eat all of it, and the excess remains on top of the
culture as a loose mixture, to drop out when one attempts to remove the
flies that have hatched. Although the culture method is by no means perfect as yet, it is adequate and reliable for present purposes.

3. Temperature conditions

Sciara is resistant to cold; the only effect of low temperature seems to be a retardation of the rate of development. The larvae are very sensitive to heat, however, and 29°C is lethal if maintained more than a short time. Higher temperatures are immediately lethal. In the laboratory the cultures are kept in an incubator with a temperature range of 22°-24°C. Moisture conditions are regulated by placing a large flat pan of water on the lowest shelf in front of an electric fan which is in continuous operation. Under these conditions the life cycle of *S. coprophila* occupies about a month, divided approximately as follows: egg stage 5–6 days; larva 14–15 days; pupa 3–4 days; adult 5–8 days. Twelve to fourteen successive generations may be grown in the course of a year.

4. Breeding technique

The type of inheritance found in Sciara necessitates certain variations from the usual breeding technique employed with other animals. *S. coprophila* is "monogenic," individual females typically giving "unisexual" progenies. (One bisexual line arose as a mutation (Metz 1931) and is being studied). Occasionally there will be one or more “exceptional” males in a female progeny, or “exceptional” females in a male progeny, in which case sib matings can be made, but usually such inbreeding is not possible and it cannot be relied on as a method of studying linkage. The precise methods employed will be given more fully in the section on linkage.

In maintaining mutant stocks in the laboratory it has not proved feasible to combine several characters in one stock as is done in Drosophila work. When this has been attempted, the stocks have lost viability, despite every care. For this reason pedigreed stocks of each line have been kept. In practically all the work pair matings are used in maintaining stocks, mutant females being out-crossed to wild type males from a wild stock every generation to keep the lines viable. Even with these precautions the mutant stocks frequently show poor viability. The wild stocks also show considerable variation in this respect. These fluctuations do not appear to be related to any immediate environmental effect, for it rarely happens that more than one stock is in poor condition at a given time, although all the flies are kept under the same conditions. Likewise fluctuations in viability are not usually associated with seasonal changes.

**GENERAL ACCOUNT OF THE MUTANT CHARACTERS AND THEIR OCCURRENCE**

It is exceedingly difficult to secure satisfactory mutant characters in Sciara. This is due in part to the remarkable resistance to radiation shown
by these flies, and in part to the physical characteristics which conceal all except the most obvious changes. Furthermore, the type of inheritance in this species tends to conceal recessive characters because the progenies are essentially unisexual and consequently sib matings are rare. Finally, the selective segregation occurring in the male prevents the transmission of paternal characters through the male line.

The normal rate of appearance of mutant characters in nature is very low, as witnessed by the fact that in hundreds of cultures of *Sciara coprophila* derived from five different localities and cultivated in the laboratory for a number of years in both pair matings and mass matings, only three mutant characters were found prior to 1930. All of these were recessives; two were sex-linked (swollen and narrow) and one was autosomal (truncate). During the course of the present study, two more sex-linked recessive characters (miniature and round) and two more autosomal characters (Delta, a dominant; oval, a recessive) have arisen spontaneously. Other mutant characters may have been spontaneous in origin, but since they came from lines subjected to X-ray treatments this cannot be concluded with certainty.

In an effort to increase the mutation rate, adult flies of both sexes were X-rayed. The treatments were given at the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York, and at the Marine Biological Laboratory, Woods Hole, Massachusetts. In all cases a tungsten target was used and a 1 mm aluminum filter for intercepting the soft rays. In the work done at Cold Spring Harbor the dosage was determined by means of a Victoreen Dosimeter, while at Woods Hole mathematical calculation was employed.

*Sciara* is able to withstand very high dosages of X-rays as compared with most other organisms, and shows no effect from dosages commonly employed in Drosophila work (1,000 to 5,000 r units). The range of treatments found to be effective was from 7,000 to 20,000 r units. It is not certain that the X-ray treatments actually induced the mutations. However, mutations did occur in cultures that had been treated.

It is usually assumed that it is not profitable to work with dosages which produce greater than 50 percent sterility, but in a form in which mutation is rare and which is relatively resistant to artificial means of producing mutant characters, it is feasible to use dosages which cause complete sterility in as many as 70 percent of the cases. The percentage of sterility produced is extremely variable from time to time, even when the X-ray treatment is essentially the same and the flies are similar.

Although some of the mutant characters came from treated cultures, the genetic data make it seem probable that these as well as the other mutations represent actual gene changes rather than chromosome abnormali-
Each new mutant character was tested with respect to constancy, selective segregation and linkage. All the new characters described here are dominant wing peculiarities. One was found by Mrs. C. S. Maurice, the remaining five by the writer. Their characteristics may be readily observed by examination of the photographs and comparison with the wild type wing shown in figure 3a.

In describing variations from the wild type, the terminology of wing venation used by Johannsen (1909) is followed (fig. 2).

![Diagram after Johannsen, showing Sciara wing venation](image)

Figure 2. Diagram after Johannsen, showing Sciara wing venation:

- \( H \) = humeral cross-vein at base of wing
- \( R \) = radius
- \( R_1 \) = first branch of radius
- \( R_2 \) = posterior branch of radius
- \( M \) = media
- \( M_{1+2} \) = anterior branch of media
- \( A \) = anal vein
- \( R_1 - R_2 \) = cross branch of radius
- \( M_2 \) = posterior branch of media
- \( Cu \) = cubitus
- \( Cu_1 \) = anterior branch of cubitus
- \( Cu_2 \) = posterior branch of cubitus
- \( r - m \) = radio-median cross vein

1. Curly

a. Origin. Curly is an autosomal dominant, found April 15, 1930 in a single \( F_2 \) female from X-rayed + male. Since the character is a dominant, it is probable that it would have appeared earlier had it been the result of the X-ray treatments.

b. Description. The wing appears to have expanded normally and then become curled forward from the posterior end of the wing toward the head (fig. 3b). It is extremely variable, ranging from a barely perceptible bend in the wing to an extremely tight curl. There is no irregularity of venation or unusual pigmentation. If the curl is very extreme, the wing appears to be distorted and can be flattened out only with effort. This sometimes makes classification difficult when crosses are made involving other wing characters.

c. Occurrence. Not only is Curly variable in appearance, but it is inconstant as well; it does not always show when the gene is present. In a series of tests crossing virgin females heterozygous for Curly to wild type males from stock, the progeny from 322 pair matings were: 17,371 wild type and 8,161 Curly. That is to say, of 25,532 offspring 68 percent were wild-type
Figure 3. Photographs of Sciara showing:

(a) normal or wild-type wing
(b) Curly fly, whole mount
(c) Blister wing
(d) Delta wing
(e) Fused wing
(f) Dash wing
(g) Varied wing

All photographs $\times 35$ except (b) which is $\times 5$. 
and 32 percent were Curly. This deviates widely from the 1:1 ratio which would be expected if the character were constant and of normal viability.

In a test involving 467 flies (the total progeny of four pair matings tested in another connection) Curly was found to be concealed when actually present in 17.7 percent of the flies. This fact must be taken into account in analyzing linkage values.

d. Genetic behavior of the males. Since Curly is inconstant, the transmission of maternal characters through the male is correspondingly obscured. From 18 tests in which a wild type female from stock was mated to a Curly male (the son of a Curly mother), 709 wild type and 519 Curly offspring arose, whereas, all the flies would have been Curly if the character were constant. Curly was concealed in more than 57 percent of the offspring. In four of these eighteen cases, the progeny were tested further: of the 80 apparently wild-type flies (save 13 which were infertile) each gave some Curly offspring, showing that all the wild type flies were genetically Curly.

From 29 tests of Curly males which had inherited the character from their fathers, all of the offspring were wild type except three questionable flies. One of these appeared to be Curly, but on testing was found to be wild type; the other two had rumpled wings and may possibly have been Curly, but were not tested. In stock cultures flies appear occasionally which have rumpled wings but which on breeding prove to be wild type, and since this wing characteristic is transitory, it is probably an environmental effect. It is probable that the two flies not tested belong to this class. In any case the usual genetic behavior of the Curly male is typical.

2. Blister

a. Origin. Blister is a dominant autosomal character which appeared first May 12, 1930 in a single F2 female from X-rayed wild type grand parents. If the character arose as the result of the X-ray treatments it must have been due to some latent effect, since the character, although a dominant, did not appear until the second generation after treatment.

b. Description. Blister appears as a large bubble in the general area of the posterior branching of the media vein (fig. 3c). In figure 2 this area is represented as the juncture of the media vein with M142 and M3. It looks as if the wing had failed to expand normally and that an excess of liquid had collected to form this blister-like structure. The wings of any one fly are approximately symmetrical, although the size of the blister is somewhat variable. Often the whole wing is much shrunken and distorted. There is no abnormality of pigmentation and no irregularity of venation. The size of the blister varies greatly in different flies. In the extreme form the whole middle portion of the wing is involved and is pulled out of shape, but in other cases the blister may be so slight that it produces only a swell-
ing of the media vein at the point of origin of the anterior and posterior branches. In this form it is very similar to the mutation Delta.

c. Occurrence. Blister flies are of good viability and give large progenies. 29 heterozygous Blister virgin females crossed individually to + males from stock gave 1,013 + and 846 Blister. Fifteen additional matings between heterozygous Blister females and their own Blister brothers gave 486 + and 411 Blister flies. Summarizing the results: 44 heterozygous females gave 1,499 + and 1,257 Blister flies, or a proportion of about 54 percent + to 45 percent Blister. The deficiency of the Blister class probably indicates a lower viability of the mutant flies, for on the whole the average is not far from the expected 1:1 ratio.

Blister is constant and is manifest when present, as shown by the ratios and by tests of 31 wild type sisters of Blister. The latter produced a total of 4,547 flies, of which only three were noted as questionable. One of these was tested and found to be wild type; the other two were not tested.

d. Genetic behavior of the males. Blister males derived from Blister mothers transmit the character as if they themselves were homozygous for the mutant gene. In 18 crosses of wild type virgin females by Blister males of the above type, there were 1,548 offspring all Blister.

On the other hand, Blister males which inherit the character from their fathers do not transmit it. Nineteen such males were tested by crossing to wild-type virgin females from stock. The total offspring were 1,525 wild type, and 1 questionable fly which was infertile.

3. Delta

a. Origin. Delta is an autosomal dominant character which was found June 6, 1930. It appeared simultaneously in several flies of a mass culture from pure stock of the "bisexual" line and had no history of X-ray treatment.

b. Description. Distinguishing features are relatively constant (fig. 3d). Of these, the more obvious one is the swelling of the juncture of the media vein with its anterior and posterior branches (fig. 2). The other feature is the thickening of the marginal ends of the veins termed Cui, Cu2, M1+2, and M2. Sometimes the small cross-vein (R1 - R2) is thickened noticeably. Delta is quite variable in appearance, ranging from a barely perceptible swelling of the regions designated above to a distorted, blistered wing, indistinguishable from the character Blister.

c. Occurrence. From 25 tests of heterozygous Delta females crossed to wild type males from stock, the total offspring were 1,283 + and 1,172 Delta flies. In addition, from 26 heterozygous Delta females crossed to wild type or Delta brothers which would be expected to breed pure for wild type the offspring were 1,153 wild-type and 954 Delta. Summarizing: 51 mat-
ings of heterozygous Delta females gave a total of 2,436+ and 2,126 Delta flies, or approximately 53 percent+ to 46 percent Delta. The mutant class showed poorer viability than the wild type.

On the whole the character is constant, although occasionally a fly that appears to be wild type is found to carry Delta. Eighteen wild type sisters of Delta females were tested in this connection. Of these, one gave a progeny of 167 wild type flies and one questionable fly which was dead when found. Another female gave a progeny of 142 wild type and 9 questionable flies, which when tested further were found to be actually Delta. The remaining 16 females gave only wild type offspring.

d. Genetic behavior of the males. Transmission of the character through the male line is typical of the usual inheritance found in Sciara; males transmit to their offspring only the genes derived from their mothers. From 10 Delta males crossed to wild type virgin females from stock, Delta being maternal in origin, only Delta offspring arose. Nine Delta males were tested which had inherited the character from their fathers; all gave only wild type offspring.

4. Fused

a. Origin. Fused is a dominant autosomal character which first appeared in a single female September 8, 1931. This fly was the daughter of a female which had received an X-ray treatment of 20,000 r units.

b. Description. The most distinctive feature of this character is the irregular fusion of the anterior and posterior branches of the cubitus vein (fig. 3e). This is always present, although the amount of fusion varies. The media vein is slightly irregular and appears to be the result of a puckering of the wing toward the lower margin, occasioned by the fusion of veins in that area. There may be a general increase in the amount of pigmentation of the whole wing, especially in the region anterior to the posterior branch of the radius vein (R₈) but this is not always present. The cross-vein (R₁−R₈) may be markedly thickened. In a given fly, the wings are essentially symmetrical.

c. Occurrence. Fused flies are prolific and the character is constant. Heterozygous females, when crossed to wild type males from stock give offspring that closely approximate a 1:1 ratio. Twenty-two pair matings of this type gave 1,327+ and 1,410 Fused flies, a ratio of approximately 48+ to 51 Fused. The fact that the mutant class is larger than the wild type shows the excellent viability of the mutant flies.

To test the constancy of the character, nineteen virgin wild type sisters of Fused females were crossed to wild type males from stock. These gave only wild type offspring.

An additional test was made by crossing ten wild type sisters of Fused
which were not certainly virgin (they might have mated with their wild type brother) by wild type males from stock. Nine of these gave only wild type offspring. The tenth gave only Fused offspring: 1 female and 88 males. To determine the source of Fused in these males, some were tested further; they were found to breed true for wild type. Since it is known that the males transmit only the characters derived from their mothers and these males gave only wild type offspring it is to be concluded that Fused was derived from the father, and that it was the father rather than the mother which was genetically Fused while appearing to be wild type. Apparently Fused is not often concealed for in the tests thus far made this is the only exceptional case.

d. Genetic behavior of the males. The genetic results of tests of Fused males are similar to the results obtained with other mutant characters. From 37 cases in which a wild type female from stock was crossed to a Fused male which had derived Fused from his mother, the offspring were Fused without a single exception. Likewise in 36 counts in which a wild type female was crossed to a Fused male that had received the character from his father, all of the offspring were wild type. Again the males are shown to breed as if homozygous for characters of maternal origin.

5. Dash

a. Origin. Dash is an autosomal dominant character which first appeared October 3, 1931. The first fly was a male, the son of a female which had been treated with 20,000 r units of X-rays. This male was mated successively to four different females and all the offspring showed the character. In view of further tests with this character and its transmission through the male, there can be no doubt that the mutant gene was derived from the female parent.

b. Description. The most obvious and constant characteristic is the presence of a very pronounced structure between the anterior and posterior branches of the cubitus vein (fig. 3f). This resembles the usual wing veins in color and in sharpness of outline. Its direction of slope is downward from the posterior branch of the cubitus vein toward the anterior branch. The spot is elongate and slender, variable in size, and sometimes attached at one end to the posterior branch of the cubitus vein, in which case it gives the appearance of an extra vein. More often it exists as a separate structure lying between the two veins.

Occasionally, the character is more extreme for several generations, due perhaps to the presence of undetected modifiers. This variation is characterized by the presence of numerous scattered blobs of pigment, suggesting the character Varied. Despite the altered appearance, the spot between the two branches of the cubitus vein remains distinct. Usually the Dash wing
has a cloudy appearance, with a greater amount of pigmentation in the anterior region near the wing margin.

c. Occurrence. Dash affords no exception to the usual type of inheritance found in Sciara. The character is constant and the flies are viable. 23 crosses of heterozygous Dash females by wild type males from stock, gave 1,131 and 1,150 Dash. Seven crosses of heterozygous Dash females by wild type brothers (which would breed pure for wild type) gave a total of 282 and 291 Dash offspring. Summarizing: 30 heterozygous females gave 1,413 wild type and 1,441 Dash flies, about 49 percent of the total being wild type and 51 percent Dash—approximately a 1:1 ratio.

d. Genetic behavior of the males. Transmission of the character through the male differs in no way from that observed previously in Sciara. Twelve Dash males which had received the character from their mothers were tested; their offspring without a single exception were Dash. Fourteen Dash males which had received the character from their fathers were tested; their offspring were all wild type.

6. Varied

a. Origin. Varied is a dominant, autosomal character which appeared first October 14, 1931 in one male, the son of a female which had been subjected to 13,000 r units of X-rays. This male was bred to several females and transmitted the character to all of his daughters and all but three of his sons. In view of the usual genetic behavior of Sciara males, the fact that practically all the offspring received the character (the three exceptional flies probably being Varied since the character has been shown to be inconstant), indicates that the mutant gene came originally from the treated female.

b. Description. This character is extremely variable in appearance. Figure 38 shows a characteristic wing. The chief feature is a general duskiness of color accompanied by scattered blobs of material resembling vein fragments. These spots are variable in size, shape, number and location. There may be several such fragments, well-distributed over the wing surface, or there may be only one, in which case it is usually confined to the region between the anterior and posterior branches of the cubitus vein. Often there is an intermittent fusion of the posterior and the first branches of the radius vein, but there is no regularity about this, either as to the amount or place of fusion. In its most simple manifestation the character looks very much like Dash, described previously. The two wings of a given fly are seldom symmetrical, although both wings usually show the same degree of modification.

c. Occurrence. Flies showing the character Varied emerge late in any culture, after the majority of the wild type flies have hatched. The division
of the hatching period into two phases is especially striking in female progenies derived from heterozygous mothers. If the number of offspring is small, it frequently happens that only the wild type flies hatch. Eighteen heterozygous Varied females crossed to wild type males from stock gave 1,082+ and 521 (or 32 percent) Varied. Eleven additional progenies were tested and the offspring which appeared to be wild type were tested further to determine their genetic constitution. The initial count was: 17 wild type (which were dead when found); 681 wild type (which were tested); 255 Varied. After testing the final result was found to be: 543 wild type and 410 Varied. The 17 dead flies and the flies which gave no offspring were classed as wild type in the final count, weighting the results in the direction of that class. Even with this possibility of error, 43 percent of the total offspring were found to be Varied. Of the 410 Varied flies in the final count, 155 (37 percent) had appeared to be wild type before being tested.

d. Genetic behavior of the males. As in the cases previously described, the males transmit only the genes of maternal origin, although Varied does not always show when the gene is present. From 25 pair matings (wild type virgin females crossed to Varied males from Varied mothers), the progeny were 151 wild type and 2,377 Varied. Although most of the offspring showed the Varied character as expected, about 6 percent appeared to be wild type. The latter were probably genetically Varied.

In testing the transmission of Varied through males which had derived the character from their fathers, 20 pair matings were made. Eighteen of these gave only wild type offspring as expected, but the remaining two showed unusual behavior. One gave only wild type offspring save for three females which appeared to be Varied (not tested). The second gave only wild type offspring save for one male which was Varied and which was crossed successively to 5 wild type virgin females from wild stock. One female gave no offspring. Three females gave female progenies in which every fly was Varied, indicating that the Varied male transmitted only the character of paternal origin. The remaining female gave 44 Varied females and 1 wild type male. (This male was tested but gave no offspring.) Many of the Varied females from these four progenies were tested; they gave progenies typical of the usual heterozygous females. Although a stock derived from this source was kept in the laboratory for almost two years, neither females nor males showed any further tendency to breed differently from flies of the regular Varied stock.

The appearance of these exceptional flies at once suggests contamination, a possibility which cannot be ruled out completely. However, if contamination had occurred through the entrance of a Varied male (Varied of maternal origin) into the cultures, as the results suggest, a larger number of Varied flies should have appeared. Each progeny consisted of more
than 100 flies, yet in the first exceptional case only three unusual flies arose, and in the second case only one.

The fact that Varied is inconstant, particularly in male progenies, further complicates the situation, but as they stand, the results suggest that this exceptional case may have come about as the result of a reverse segregation of chromosomes at the first spermatocyte division, which resulted in the retention of the chromosome of paternal origin, rather than the chromosome of maternal origin. If so, the male in this instance showed reversal of the usual type of inheritance found in Sciara.

**LINKAGE**

The primary purpose of the linkage tests is to identify the different chromosomes and to ascertain whether or not they all exhibit the same type of segregation. Unfortunately the type of inheritance involved here presents certain difficulties for the study of linkage because it is necessary in each case to back-cross individuals of the sex in which crossing over occurs. In spite of this difficulty, it is believed that the evidence warrants the tentative conclusion that all three autosomes have been identified.

When the present study was begun, two linkage groups were already known, one representing the sex chromosomes and the other representing the first pair of autosomes, the latter identified by the character truncate. Since none of the new characters was sex-linked, it was obvious that they did not belong to Group I.

Since backcrosses of heterozygous males are unsuitable for linkage tests because of selective segregation of chromosomes occurring during spermatogenesis, such tests had to be made in the manner indicated by the following examples. In testing the recessive character truncate with a dominant such as Blister, truncate females were crossed to Blister males (from Blister mothers). The hererozygous daughters were mated to truncate males from pure stock and the progeny counted. In testing two dominants together such as Dash and Blister, Dash females were mated to Blister males (from Blister mothers); the heterozygous daughters were outcrossed to wild type males from wild stock and the progeny were counted. Reciprocal crosses were made in both types of tests.

The results of the linkage experiments are summarized in table 1. Curly proved to be the only character representing the second pair of autosomes (Group III) and had thus to be used in spite of its inconstancy.

1. **$C \times ir$**. Curly was first crossed with the recessive truncate. The two characters did not appear to be linked, but the results were obscured by the inconstancy of Curly. Since the required data were important, the tests were repeated and all the questionable flies were tested further. This entailed the testing of all wild type flies for Curly, all truncate flies for
Curly, extreme Curly flies for truncate, and shrivelled flies for both truncate and Curly. A small number of weak flies were dead soon after hatching and could not be tested further. Some of the flies that were tested proved to be infertile. In the final analysis the flies of these two groups were included in whatever class they appeared to belong.

There is no doubt some error in the final calculations due to the fact

<table>
<thead>
<tr>
<th>CASES</th>
<th>TOTAL FLIES</th>
<th>PERCENTAGE CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. C×tr</td>
<td>15</td>
<td>2,009</td>
</tr>
<tr>
<td>2. C×B</td>
<td>35</td>
<td>1,855</td>
</tr>
<tr>
<td>3. B×tr</td>
<td>44</td>
<td>3,666</td>
</tr>
<tr>
<td>4. C×Δ</td>
<td>29</td>
<td>2,727</td>
</tr>
<tr>
<td>5. Δ×tr</td>
<td>35</td>
<td>3,985</td>
</tr>
<tr>
<td>6. B×F</td>
<td>30</td>
<td>2,345</td>
</tr>
<tr>
<td>7. F×Δ</td>
<td>13</td>
<td>650</td>
</tr>
<tr>
<td>8. F×tr</td>
<td>11</td>
<td>565</td>
</tr>
<tr>
<td>9. F×C</td>
<td>8</td>
<td>361</td>
</tr>
<tr>
<td>10. D×B</td>
<td>16</td>
<td>890</td>
</tr>
<tr>
<td>11. D×Δ</td>
<td>10</td>
<td>436</td>
</tr>
<tr>
<td>12. D×tr</td>
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<td>701</td>
</tr>
<tr>
<td>13. D×C</td>
<td>13</td>
<td>766</td>
</tr>
<tr>
<td>14. V×tr</td>
<td>43</td>
<td>2,847</td>
</tr>
</tbody>
</table>

+ indicates wild type.

Detailed data from which this table was prepared have been placed on file with "Genetics" and may be consulted by anyone interested.

that the classification of the infertile flies, of necessity, followed the original classification given them. This classification was probably correct in many cases, but the extent of accuracy cannot be known. There were in this group 111 normal, 105 truncate, 9 Curly, and 1 fly classed as wild type which may have been truncate, (it was classed as shrivelled at first but was tested only for Curly). If some of these infertile wild type flies were actually Curly, the Curly class would be larger at the expense of the wild
type class; and likewise, if some of the infertile truncate flies were Curly, the Curly-truncate class would be larger, with a corresponding diminution of the truncate class. The few infertile Curly flies might have been either Curly or Curly-truncate. There were 226 of these questionable flies which comprised 11 percent of the total offspring.

The offspring from 15 pair matings were tested with the results shown in table I (1). The total number of flies involved was 2,009, divided approximately as follows: 25 percent wild type, 23 percent truncate, 29 percent Curly, and 22 percent Curly-truncate. The conclusion seems warranted that Curly and truncate are not linked since each pair mating showed a clear division of the progeny into four approximately equal classes, even in the tests which had the greatest number of infertile flies. Since truncate was previously assigned to the first pair of autosomes, on the basis of earlier evidence, Curly can now be regarded as located in the second pair of autosomes (Group III), pending further analysis.

2. C×B. Turning to table I (2), the counts of 35 tests of Curly by Blister gave 1,855 flies of which 37 percent were wild type, 25 percent Blister, 18 percent Curly, and 17 percent Blister-Curly. Since Curly is inconstant, it is evident that a number of flies classed as wild type must have been genetically Curly, and likewise a number classed as Blister were genetically Blister-Curly. This accounts, at least in part, for the size of the wild type and Blister classes as compared with the Curly classes. The concentration of the averages into the wild type and Blister classes does not suggest that the characters are linked, for if they were, the Blister and Curly classes would be expected to be the largest and the wild type and Blister-Curly classes the smallest. This interpretation receives further support from the evidence presented in the following paragraph.

3. B×tr. Table I (3) shows the results of 44 tests of truncate by Blister, involving 3,666 flies. Thirty-four of these were shrivelled; the remainder were approximately 29 percent wild type, 20 percent truncate, 43 percent Blister, and 6 percent Blister-truncate. The most interesting feature here is the excessive number of Blister flies. The probable explanation is that extreme Blister causes a great distortion of the wing so that the whole shape is askew; truncate affects only the shape of the wing; when the two characters are present in the same wing, the presence of Blister so alters the shape of the wing that truncate is concealed. Furthermore, it is the Blister-truncate class which is deficient throughout and the Blister class which is in excess. If some of the flies classed as Blister were actually Blister-truncate, these two groups would tend to become more nearly equal. If the Blister-truncate group represented a crossover class, it would be expected that the wild type class also would be small, which is not the case. Blister is not linked to either truncate or Curly, so tenta-
tively at least, it can be stated that Blister is in the third pair of autosomes (Group IV). Thus each pair of autosomes is represented by a character, and it remains to be shown in which linkage groups the remaining four mutant characters belong.

4. **C×A.** Delta was studied first in its relation to Curly with the results set forth in table 1 (4). From 29 pair matings 2,727 offspring arose. Twenty nine percent were wild type, 28 percent Delta, 18 percent Curly, and 24 percent Delta-Curly. Here the Curly and Delta-Curly classes are smaller than the wild type and Delta classes. Again it must be emphasized that the inconstancy of Curly obscures the true results. If the Curly gene were present in some of the wild type and some of the Delta flies, as was probably the case, the Curly and Delta-Curly classes would be proportionately increased. This would tend to establish the $1:1:1:1$ ratio expected in characters that are not linked.

5. **A×tr.** Delta was next tested with truncate, as shown in table 1 (5). The 3,985 offspring from 35 pair matings were approximately as follows: 25 percent wild type, 25 percent truncate, 26 percent Delta, and 22 percent Delta-truncate. Twelve shrivelled flies were not included in the above averages. The flies showing both mutant characters evidently have a lower viability than those of the other classes, for on the average, the Delta-truncate class is the smallest. This is probably not due to any difficulty in classification, for both characters are definite and easily recognized, even when present in the same wing. Truncate and Delta are relatively constant; the percentage of cases in which the characters are concealed is too small to explain the deficiency of the Delta-truncate class on that basis. Furthermore, the three other classes, (wild type, truncate, and Delta) are very nearly equal in size and show no undue concentration of flies in any one class. In three of the matings the progeny were divided into classes almost equal numerically. Evidently Delta and truncate are not linked.

6. **A×B.** Having found that Delta was not linked either to Curly (Group III) or truncate (Group II) the inference was that it must be linked to Blister (Group IV). The proof of this by crossing Blister with Delta was difficult, chiefly because the two characters are so similar that a double mutant class, if it did arise, would never be distinguishable as such. In addition, the extreme Delta wing so closely resembles Blister that a classification of these groups could never be accurate. A test to secure the information indirectly was made in the following manner. Blister virgin females were crossed to Delta males, the sons of a Delta female. (The reciprocal test was also made.) From individual female progenies arising thus, 26 of the most extreme flies, which might be both Blister and Delta, were outcrossed to wild type males from stock. If any of the females were Blister-Delta, this should be apparent in the progenies. Without exception,
however, there were only two types of offspring from each mating; these were either wild type and Blister or wild type and Delta; never were there three classes. Such results suggest that the two characters can be combined in one fly only with difficulty if at all, and that perhaps they are allelo-
morphs. This impression is further strengthened by the fact that it is
difficult, if not impossible to get either of these characters in a homozygous
condition.

7. $B \times F$. Fortunately the linkage of Blister and Delta can be shown
through a study of their respective relationship to another character,
Fused. The results of crossing Blister with Fused are shown in table 1 (6)
The progeny fall into two major classes, Blister and Fused, in almost equal
numbers. Of the total offspring from 30 pair matings, 46 percent are Blister
and 46 percent Fused. The cross-over classes are exceedingly small, aver-
ging approximately 3 percent wild type and 4 percent Blister-Fused. Not
only is the linkage apparent from the averages of all cases, but the same
type of result is characteristic of each mating throughout the whole series.
In no case is there a grouping of the progeny into four approximately
equal classes.

8. $\Delta \times F$. The results of crossing Delta with Fused are similar to those
obtained from crossing Blister with Fused. Although the number of tests
is less and the averages are smaller, the grouping of the progeny into two
major classes is definite, whether viewed from the standpoint of individual
matings or of the combined averages. The results are given in table 1 (7).
From 13 pair matings the progeny are divided as follows: 6 percent wild
type, 35 percent Delta, 56 percent Fused, and .9 percent Fused-Delta. In
some individual matings the Fused and Delta classes are about equal
numerically, but on the whole the Fused class is considerably larger. This
probably indicates a poorer viability of Delta.

9. $F \times tr$. That Fused appears to be linked to both Blister and Delta is fur-
ther supported by the fact that it appears to be independent of both trun-
cate and Curly. The results of the Fused-truncate crosses are given in table
1 (8), the averages for 11 pair matings being 28 percent wild type, 20 percent
truncate, 31 percent Fused, and 20 percent Fused-truncate (5 shrivelled
flies omitted). In some of these matings the truncate and truncate-Fused
classes are smaller than the wild type and Fused classes, which in turn
affects the final averages for these groups. Since the results are not typical
of linkage (if the characters were linked the truncate and Fused classes
would be expected to predominate), it is probable that the poor viability
of truncate is responsible. In three of the eleven pair matings, the progeny
were divided into four classes of almost equal size.

10. $F \times C$. The final counts of crossing Fused with Curly (table 1 section
9) indicate, when the inconstancy of Curly is considered, that the two
characters are not linked. From 8 pair matings the count was 32 percent wild-type, 15 percent Curly, 34 percent Fused, and 17 percent Fused-Curly. Doubtless some of the wild type flies were genetically Curly and some of the Fused flies were actually Fused-Curly. In summary it may be said that Blister, Delta, and Fused have been shown to be linked and to represent the third pair of autosomes (Group IV).

11. $D \times B$. Dash was crossed to Blister and to Delta with results essentially alike in both cases, again confirming the linkage of Blister and Delta. In Table 1(10) the averages of 16 crosses of Dash and Blister are: 29 percent wild type, 41 percent Blister, 21 percent Dash, and 7 percent Blister-Dash. The Dash class is much smaller than either the wild type or Blister classes, due probably to poor viability. The small size of the double mutant class would suggest linkage were it not for the fact that the wild type class (which would be the other cross-over class if this were the case) is so large. Probably the flies having two mutant genes were extremely weak and many failed to hatch.

12. $D \times \Delta$. The results of crossing Delta with Dash are given in table 1(11). Again the two Dash classes are smaller than the wild type and Delta classes but the differences are not as large as in the Dash and Blister crosses. The progeny from 10 pair matings are: 30 percent wild type, 25 percent Delta, 22 percent Dash, and 22 percent Delta-Dash. Dash could not be tested with Fused because of the similarity of the two characters.

13. $D \times tr$. The characters Dash and truncate were found to be linked. Table 1(12) shows that there are only two main classes of progeny, truncate and Dash in the backcrosses. The 701 offspring from 15 pair matings are: 34 percent truncate, 62 percent Dash, with the crossover classes represented by only 2 percent wild type and .8 percent Dash-truncate. Two shrivelled flies are not included. On the whole, truncate seems to be less viable than Dash, for there are almost twice as many Dash as truncate flies. The truncate class is deficient in the matings where the total number of progeny is small. In five of the fifteen matings the progenies are fairly large; in these, truncate and Dash are present in about equal numbers. The crossover classes are almost non-existent.

14. $D \times C$. On the basis of the tests described above (11, 12, and 13) Dash was thought to be located in chromosome Group II. To verify this conclusion it was tested with Curly. It was anticipated that the two characters would be found to be independent of each other, but since a study of the relationship between Dash and Curly would provide additional information concerning the relationship of truncate and Curly (truncate being linked with Dash), the analysis was undertaken in some detail.

The results of the first tests of Dash and Curly are as follows: 13 cases
gave 1 percent shrivelled, 33 percent wild type, 23 percent Curly, 30 percent Dash, and 10 percent Dash-Curly. Since the inconstancy of Curly proved such an obstacle to interpretation of these data, a second test was made in which the non-Curly flies from 5 pair matings were tested further. The initial count was 7 percent shrivelled, 39 percent wild type, 24 percent Curly, 18 percent Dash, and 10 percent Dash-Curly. After testing, however the percentages became: 1 percent shrivelled, 24 percent wild type, 24 percent Curly, 6 percent Dash, and 43 percent Dash-Curly. The infertile flies and the dead flies which could not be tested were tabulated in whatever class they appeared to belong, thus unavoidably introducing some degree of error. This group comprised 13 percent of the total offspring. In the revised count there was an unusually large number of Dash-Curly flies, due probably to the presence of unidentified modifiers which affected Dash to some extent, causing many Dash wings to resemble also the least extreme form of Curly. Since this type of variation frequently occurs and since it was present in pure Dash stock at the time these experiments were conducted, this explanation seems plausible.

The results of the second test were analyzed further, omitting all doubtful flies, and considering only the flies of which the true classification was known. Of 171 wild type flies tested, 42 percent proved to be genetically Curly. Of the 122 Curly flies, 62 percent proved to be Dash-Curly. Correcting the results of the first Dash×Curly experiment on the basis of these percentages, (transferring 42 percent of the wild type class to the Curly class and 62 percent of the Curly class to the Dash-Curly class) the final averages become: 20 percent wild type, 23 percent Curly, 31 percent Dash and 24 percent Dash-Curly. The "corrected" averages are shown table 1(13).

Although there is some inaccuracy in drawing conclusions from such indirect analysis, it seems clear that Curly and Dash are not linked, as the wild type and Dash-Curly flies are too numerous to be considered as crossover classes. These data also further support the conclusion that Curly is not linked to any of the other mutant characters and hence represents the only gene thus far identified in the third pair of chromosomes (III).

15. V×tr. It is impossible to study the linkage of Varied with any character save the recessive truncate, because Varied has certain features in common with all the others and accurate classification of the progenies would be impossible. Fortunately the tests of Varied with truncate provide all the data needed. As mentioned previously, Varied is not constant; some flies in Varied cultures appear to be wild type when they are genetically Varied. For this reason, all the flies classed as wild type in the Varied-truncate crosses were tested further to verify their classification. In these tests, Varied hatched late in every mating; practically all the truncate
flies hatched before Varied flies began to hatch. This is typical of the Varied cultures in general.

As shown in table 1(14), 43 pair matings were studied. In 23 cases there were no wild type flies to be tested. Of the 53 wild type flies tested more than half were found to be Varied. In the final calculation, among 19 wild type flies, 5 were known to be wild type, 2 were probably wild type but possibly Varied, and the remaining 12 were infertile but were classed as wild type because of their appearance. This whole group formed less than .6 percent of the total progeny. The Varied-truncate group also was exceedingly small, the total number of flies being 18, (less than .6 percent of the total progeny). The flies were mainly of two types, truncate and Varied, the averages being 57 percent truncate and 40 percent Varied. (Only the final averages are shown in table 1). It seems evident from these results that Varied belongs to the truncate-Dash linkage group (II).

Among the tests of wild type females from this series of experiments, four of the matings gave unusual results. These wild type females, heterozygous for truncate, were outcrossed to wild type males from wild stock 2567. The progeny which were expected to include only wild type and Varied contained some truncate flies as well. Further analysis showed that the 2567 stock was contaminated with truncate; the appearance of truncate in these further tests with Varied-truncate was undoubtedly due to this factor rather than to any unusual behavior of the Varied and truncate lines.

SUMMARY

(1) Six new wing characters in *Sciara coprophila* have been studied, all of which are autosomal dominants.

(2) Each character has been shown to segregate selectively in the male so that only characters of maternal origin are transmitted.

(3) A study of linkage has shown that all (except the "limited") chromosomes have now been identified by means of mutant genes and that accordingly the autosomes may be designated as follows:
   - Chromosome II—truncate, Dash, Varied
   - Chromosome III—Curly
   - Chromosome IV—Blister, Delta, Fused

   It is not known which pair of chromosomes corresponds to any particular linkage group cytologically, except in the case of the sex chromosome. Since all of the autosomes are paired in the male soma, it is evident that the unpaired chromosome is not an autosome but is the sex chromosome.

(4) The rate of crossing-over for the autosomes was found to be very small, which corresponds to the results obtained previously for the sex chromosomes (Metz and Schmuck 1931, Metz and Smith 1931).
SELECTIVE SEGREGATION IN SCIARA

ACKNOWLEDGMENTS

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