A NEW SEX-RATIO ABNORMALITY IN *DROSOPHILA OBSCURA*[*]

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MATERIALS AND METHODS

Drosophila obscura Fallén is commonly found in the neighbourhood of Moscow. Apparently, it in no way differs from the American fly; at least, the coincidence between all the morphological characters of both species is complete. The cytological investigation of the Russian *D. obscura* is unfinished as yet; some preliminary data, which S. L. FROLOVA most obligingly communicated to me, mark the presence of a long V-shaped X-chromosome, perfectly distinct from the rodlike Y-chromosome. The American *D. obscura*, according to METZ and MOSES (1923), has a bent X-chromosome which, as in the Russian species, differs distinctly from the straight and shorter Y-chromosome.

In America *D. obscura* is usually raised upon bananas; in our laboratory, in spite of many experiments, no completely satisfactory diet has yet been found. The best results are obtained with a nutritive medium prepared from a mixture of fermented raisins (100 g.), potatoes (400 g.), agar-agar (4 g.) and water (800 ccm.), but even on this medium the percent of perishing cultures is comparatively large. Also the number of offspring produced by one pair is less than that usually observed in other species of Drosophila. I could never succeed in obtaining a mating that produced more than 200 flies, and the mean number for the offspring in a culture did not rise above 40–45.

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The flies were raised in sterilized flat-bottomed glass cylinders, containing some nutritive medium lightly sprinkled with yeast; the cylinders were corked by thick cotton-wool stoppers. The cultures were kept in a temperature oscillating from 21° to 25°C. Particular attention was paid to the virginity of the females used for mating; only such were considered virgin as had yet folded wings, or had been hatched in the absence of males (for instance from isolated pupae). On an average, the development of the fly took about 20 days, but this term varied considerably, chiefly in accordance with the temperature. The flies hatched were narcotized with ether and carefully examined through a binocular microscope, all the results being recorded in detail.

TABLE 1

OFFSPRING MORE THAN 20 FLIES

NUMBER OF CULTURES	NUMBER	♂♂	♀♀	TOTAL	PERCENT ♂♂
	1	21	23	44	47.7
	3	14	16	30	46.7
	4	13	13	26	50.0
	6	26	26	52	50.0
	8	22	18	40	55.0
	9	23	19	42	54.8
	10	20	14	34	58.8
	11	21	21	42	50.0
	12	21	19	40	52.5
	13	10	10	20	50.0
	14	18	25	43	47.9
	15	17	18	35	48.6
	16	14	10	24	58.3
	18	17	15	32	53.1
	19	35	33	68	51.5
	20	13	19	32	40.6
16	..	305	299	604	50.4

OFFSPRING LESS THAN 20 FLIES

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PERCENT ♂♂
3	..	20	28	48	41.7

TOTAL

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PERCENT ♂♂
19	..	325	327	652	49.9

APPEARANCE OF THE ABNORMALITY

In July, 1925, I caught in a forest near the Hydrophysiological Station in Zvenigorod (near Moscow) 19 females of *D. obscura*. They were all fecundated already before being caught, and the offspring they gave were perfectly normal as regards the sex-ratio (table 1). Altogether 327 females and 325 males were obtained, thus giving a relation of 1.003:0.997, instead of the expected 1:1. From each culture of the first generation ten pairs of flies were inbred. In the F_2, both in the sum total and in the separate crosses the offspring of 17 of the wild females showed a normal sex-ratio. At the same time in the progeny of two females a sharp deviation from the expected relations was found. The appearance of these abnormalities is shown in figure 1. In two lines, line 3 and line 13, cultures appeared totally or almost totally consisting of females. Altogether, four such cultures were obtained (Nos. 24 and 37 in line III and Nos. 38 and 95 in line XIII). The deviations from the normal sex-ratio were so considerable that it seemed impossible to explain them by accidental causes. A further analysis of the phenomena was therefore undertaken.

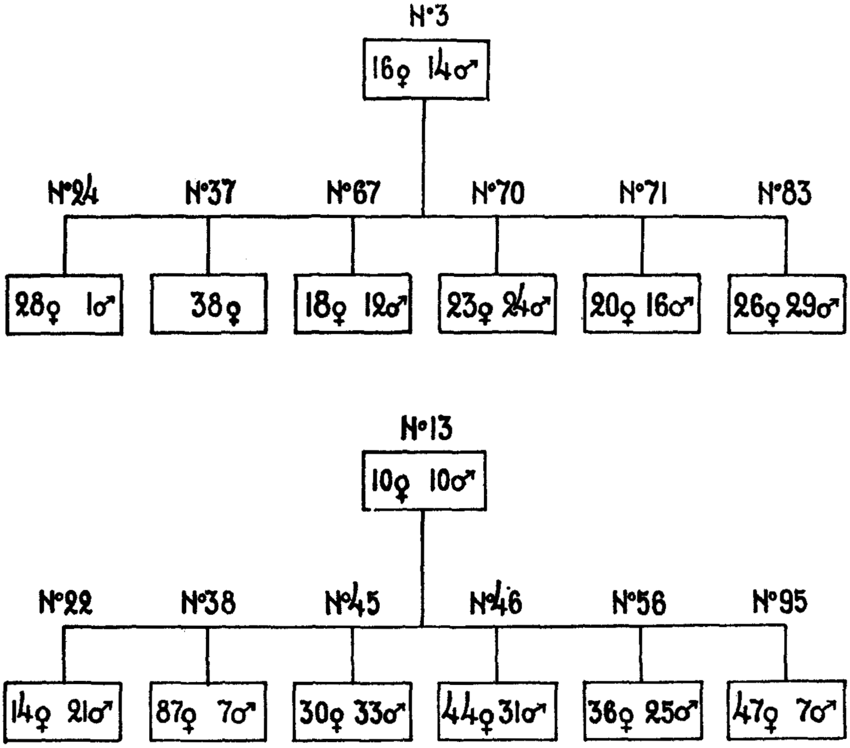

Figure 1

CHARACTERISTICS OF THE ABNORMALITY

The females resulting from the abnormal cultures of the second generation of lines 3 and 13 were mated in both lines partly with their brothers, partly with males from kindred normal cultures. The first generation proceeding from these matings showed itself everywhere perfectly normal. Further, in the succeeding generations, the offspring of these flies were bred in both lines by close inbreeding as well as more freely, within the limits of the line. A part of these crosses always showed a normal sex-ratio, while the other gave a progeny resembling, as to its results, the original abnormal cultures, namely, either consisting exclusively of females, or presenting only an insignificant number of males. Both in lines 3 and 13 the number of males in these cultures varied between 0 and 15 percent, on an average however not exceeding 4 to 5 percent of the total number of flies. Henceforth cultures showing such a predominance of females will be spoken of as "female" ones.

For several consecutive generations no more detailed investigation of the described abnormality was undertaken. During all this period I confined myself to maintaining line 3 by crossing the females from the"female" cultures either with their brothers, if such existed, or else with males from normal cultures of the same line. Also the progeny of these normal cultures was occasionally inbred. In all these cases "female" cultures were obtained as well as normal ones. As a result of all these crossings I was able to collect somewhat extensive statistical materials, which showed in line 3 of *D. obscura* the presence of but two types of sex-ratios, the normal and the "female" one, and the total lack of any transitions between them. The results are represented in figure 2. They include all the individual matings, in which the flies of line 3 participated, and which resulted in more than 20 offspring. All the matings are divided, according to the percent of males in the offspring, into classes, which are shown on the abscissa. The number of matings belonging to each class are shown on the ordinates. As can be easily seen, all the crosses fall into two independent groups. The first one includes the cultures with a practically normal sex-ratio, that is, with about 50 percent of males. This group constitutes the right curve of the distribution, which is almost regular and shows that the observed slight deviations from the expected relations are due here to accidental causes. All the other cultures form the left curve; the average number of males is here only 4.1 percent of the total number of flies and the mode of the curve lies at zero. No intermediate cultures appeared which could mar this picture.

This table can be compared with another one, constructed exactly on the same plan for the seventeen normal lines of *D. obscura* (figure 3). Figure 3 includes all the individual matings from all the normal lines, that is, from all the inbreeding lines originating from the wild females, except 3 and 13. The distribution of the cultures is here quite analogous to that presented by the right curve of figure 2; no "female" or other deviating sex-ratios are met with.

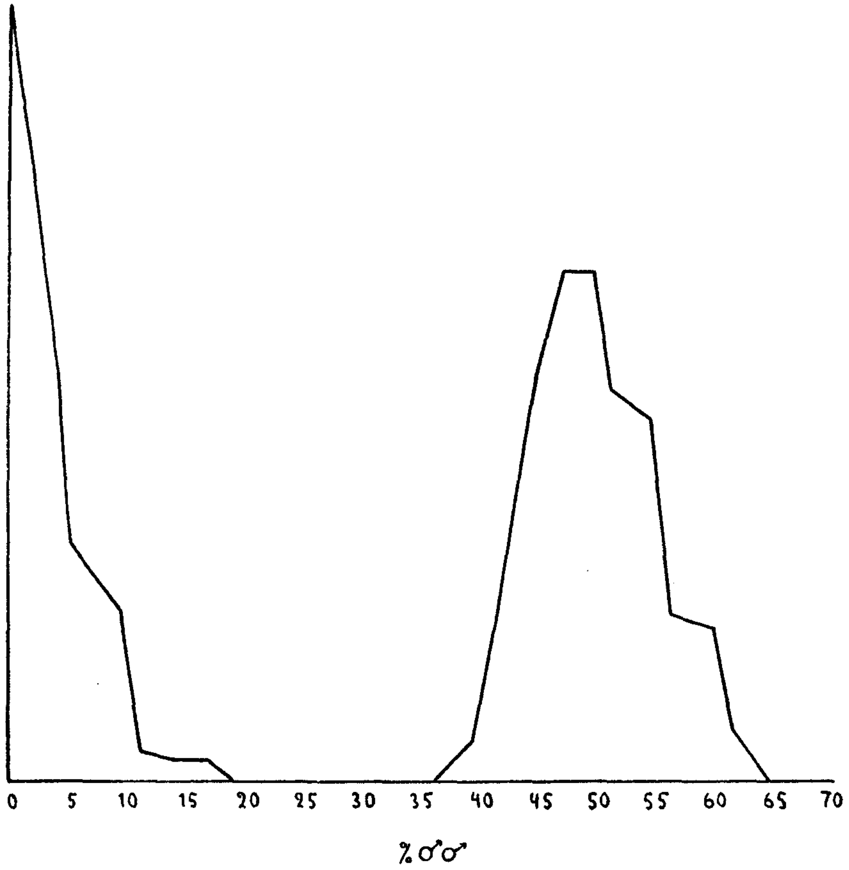

FIGURE 2.—Left curve: M=4.1; n=102. Right curve: M=49.0; n=185.

Altogether, the preliminary results of this work can be summed up as follows. The researches upon the seventeen lines, started from wild females, have made it clear that as a rule the deviations from the normal sex-ratio are in *D. obscura* very small and can be easily explained by accidental causes. The crosses in line 3 present at the same time some cultures with abnormal sex relations, the number of males being in them, on an average, only about 4 percent of the total number of flies. No transitions are observed in this line between the normal and the deviating cultures. An analogous phenomenon was observed in another line (13) while it existed.

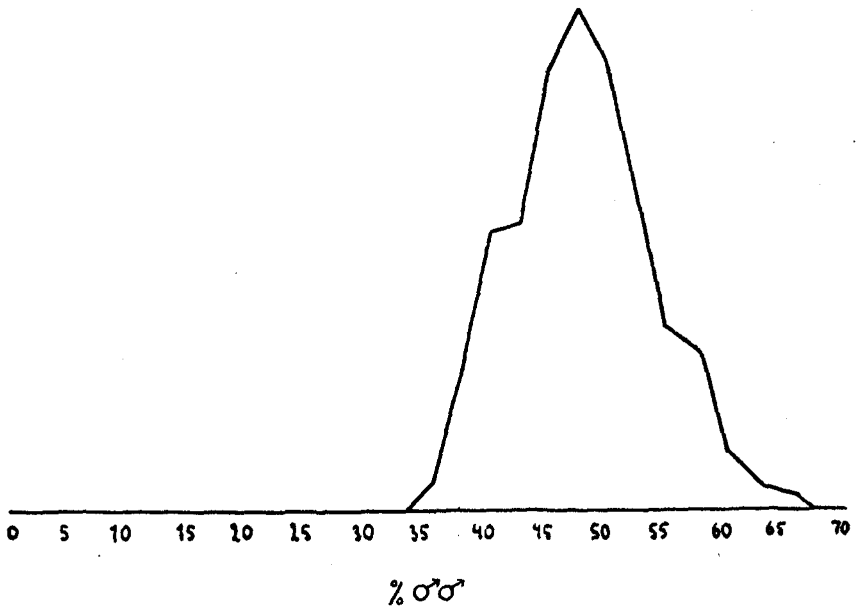

FIGURE 3.—M = 48.0; n = 194.

The data enumerated show that this abnormality is certainly hereditary, but, on the basis of these preliminary investigations it has not been possible to give its more detailed genetical characteristics; in order to do so, a whole new series of special crossings had to be organized.

GENETIC ANALYSIS OF THE ABNORMALITY

By mating a female from the "female" culture No. 1500 with its only brother, an offspring was obtained with a normal sex-ratio (49.4 percent ♂♂) and a comparatively large number of flies. This culture received the No. 1644 and served as a basis for all the subsequent investigations. Two series of crosses were organized from its offspring. The first series, designated as "type I," was produced by individual matings of virgin females from the culture No. 1644 with males of a practically pure normal line of *D. obscura*, that had passed through more than ten generations of close inbreeding without ever presenting any deviations from the usual sex proportions. All these crosses showed normal sex relations very near 1:1; there were altogether 17 crosses giving more than 20 flies and 5 crosses with a smaller number of offspring. These results are represented in table 2. The second series (type II) consisted of crosses, the reverse of the preceding ones; that is, virgin females from the pure normal line were crossed with males from No. 1644. Without any exception the crosses of this type showed "female" sex-ratios; 27 crosses were obtained, 19 of which gave more than 20 flies (table 3).

TABLE 2

OFFSPRING MORE THAN 20 FLIES

NUMBER OF CULTURES	NUMBER	♂♂	♀♀	TOTAL	PER CENT ♂♂
	1734	10	12	22	45.5
	1735	23	16	39	59.0
	1738	17	15	32	53.1
	1742	21	21	42	50.0
	1744	21	14	35	60.0
	1746	26	35	61	42.6
	1751	22	22	44	50.0
	1752	10	11	21	47.6
	1755	21	26	47	44.7
	1758	18	12	30	60.0
	1761	21	23	44	47.7
	1763	17	15	32	53.1
	1765	20	25	45	44.4
	1766	14	19	33	42.4
	1768	24	28	52	46.2
	1770	35	32	67	52.2
	1771	18	15	33	54.5
17	..	338	341	679	49.8

OFFSPRING LESS THAN 20 FLIES

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PER CENT ♂♂
5	..	19	28	47	40.4

TOTAL

NUMBER OF CULTURES	..	♂♂	♀♀	TOTAL	PER CENT ♂♂
22	..	357	369	726	49.2

TABLE 3

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1673	4	41	45	8.9
	1674	..	24	24	0.0
	1677	2	19	21	9.5
	1680	3	42	45	6.7
	1682	1	32	33	3.0
	1683	1	25	26	3.8
	1685	..	25	25	0.0
	1687	..	34	34	0.0
	1689	..	29	29	0.0
	1690	4	33	37	10.8
	1693	1	26	27	3.7
	1710	2	37	39	5.1
	1713	3	71	74	4.1
	1715	..	51	51	0.0
	1721	..	22	22	0.0
	1726	..	28	28	0.0
	1729	2	28	30	6.7
	1731	..	20	20	0.0
18	..	23	587	610	3.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
9	..	2	97	99	2.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
27	..	25	684	709	3.5

The offspring of the crosses of type I did not undergo any further analysis. As to type II, two series of crosses were again started from its cultures. One series, named type III, consisted of crosses between virgin females from culture No. 1713 (of type II) and males from the pure normal line. All these crosses gave normal offspring; table 4 presents the numbers obtained. The other group included crosses between normal virgin females and the males out of all those cultures of type II, where they existed in the

TABLE 4

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1820	20	22	42	47.6
	1821	21	16	37	56.8
	1823	30	21	51	58.8
	1826	14	20	34	41.2
	1827	25	24	49	51.0
	1828	48	51	99	48.5
	1829	14	15	29	48.3
	1835	21	25	46	45.2
	1838	15	23	38	39.5
	1840	18	18	36	50.0
	1841	14	17	31	45.2
	1845	26	26	52	50.0
	1846	18	19	37	48.6
	1851	43	52	95	45.3
	1853	16	20	36	44.4
	1854	19	20	39	48.7
	1856	19	16	35	54.3
	1859	21	28	49	42.9
18	..	402	433	835	48.1

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
6	..	25	28	53	47.2

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
24	..	427	461	888	48.1

offspring. Nineteen such crosses (named type IV) were obtained, all of them normal in regard to the sex-ratio (table 5). Lastly, from the offspring of the culture of type III two more types of crossings were organized: the crossings of virgin females from the culture No. 1828 with males of the normal line (type V), and reversed crossings of normal females with males from the cultures of type III (type VI). All the matings of the V type presented normal sex-relations (table 6). On the contrary, in type VI "female" cultures again appeared.

TABLE 5

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	1861	14	16	30	46.7
	1865	15	11	26	57.7
	1866	20	25	45	44.4
	1871	24	27	51	47.1
	1873	20	23	43	46.5
	1875	16	22	38	42.1
	1876	12	12	24	50.0
	1880	13	17	30	43.3
	1882	18	18	36	50.0
	1883	34	28	62	54.8
	1885	10	11	21	47.6
	1887	12	10	22	54.5
	1888	29	20	49	59.2
	1890	26	19	45	57.8
	1896	13	14	27	48.1
15	..	276	273	549	50.3

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
4	..	16	25	41	39.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
19	..	292	298	590	49.5

The crosses of type VI, though alike in structure, fall into two groups owing to the origin of the participating males: 27 cultures were obtained from the mating of males from the culture No. 1828 with normal virgin females, while 37 cultures resulted from analogous matings of males from culture No. 1851 (also of type III). Both in the first and in the second group "female" cultures appeared, and in both cases very nearly one half of the matings gave a "female" progeny (table 7 and table 8). Altogether 47 matings were obtained (leaving out those which produced less than 20 flies): 23 matings gave a normal offspring, and 24 matings presented typically "female" sex-ratios. Thus the relation between the number of normal matings and of "female" ones was found to be in type VI very near to 1:1 (table 9).

Table 6

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2021	10	15	25	40.0
	2023	26	21	47	55.3
	2027	24	17	41	58.5
	2028	19	17	36	52.8
	2032	16	12	28	57.1
	2035	24	30	54	44.4
	2036	11	9	20	55.0
	2037	14	13	27	51.9
	2045	13	12	25	52.0
	2048	19	22	41	46.3
	2051	18	24	42	42.9
	2053	16	13	29	55.2
	2054	18	19	37	48.6
	2060	14	17	31	45.2
	2061	26	26	52	50.0
	2065	10	12	22	45.5
	2066	26	22	48	54.2
17	..	304	301	605	50.2

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
6	..	28	35	63	44.4

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
23	..	332	336	668	49.7

This is the last series of matings undertaken in order to analyse the phenomenon of irregular sex-distribution in *D. obscura.* The examination of the results obtained allows us to draw some important conclusions as to the mode of inheritance of this abnormality.

First of all, wherever the females originate the progeny is perfectly normal, provided they are mated with males belonging to the pure normal line. This is distinctly to be seen in the matings of type I, III and V. Meanwhile, the matings of type II, arranged on the reverse principle,

TABLE 7

OFFSPRING MORE THAN 20 FLIES

Number of Cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2070	..	34	34	0.0
	2072	23	20	43	53.5
	2073	..	22	22	0.0
	2078	1	41	42	2.4
	2082	19	19	38	50.0
	2084	20	15	35	57.1
	2085	21	28	49	42.9
	2086	12	12	24	50.0
	2089	..	28	28	0.0
	2097	6	74	80	7.5
	2099	3	50	53	5.7
	2102	24	21	45	53.3
	2103	..	43	43	0.0
	2105	26	30	56	46.4
	2106	2	31	33	6.1
	2110	1	31	32	3.1
	2112	26	25	51	51.0
	2117	14	19	33	42.4
	2122	1	19	20	5.0
	2123	30	32	62	48.4
20	..	229	594	823	27.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
7	..	25	79	104	24.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
27	..	254	673	927	27.4

where the females were taken from the acknowledged normal line, and the males alone belonged to line III, yielded all a clearly expressed "female" offspring; the same was observed for half of the matings of type VI. This indicates that all the males from No. 1644 and half of those from cultures of type III, when mated even with pure normal females, possess the faculty of giving even in the first generation the same deviating sex-ratios as characterize line 3 of *D. obscura.*

TABLE 8

OFFSPRING MORE THAN 20 FLIES

Number of cultures	Number	♂♂	♀♀	Total	Percent ♂♂
	2125	14	14	28	50.0
	2129	4	69	73	5.5
	2131	1	34	35	2.9
	2135	18	20	38	47.3
	2136	..	36	36	0.0
	2138	23	23	46	50.0
	2145	17	11	28	60.7
	2147	1	21	22	4.5
	2148	..	30	30	0.0
	2162	..	44	44	0.0
	2164	20	24	44	45.5
	2165	24	23	47	51.1
	2166	2	41	43	4.7
	2167	..	40	40	0.0
	2174	29	23	52	55.8
	2175	11	13	24	45.8
	2178	24	26	50	48.0
	2179	1	39	40	2.5
	2180	..	20	20	0.0
	2183	2	19	21	9.5
	2184	24	22	46	52.2
	2186	17	16	33	51.5
	2191	..	35	35	0.0
	2192	18	24	42	42.9
	2903	..	51	51	0.0
25	..	250	718	968	25.8

OFFSPRING LESS THAN 20 FLIES

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
7	..	22	88	110	20.0

TOTAL

Number of Cultures	..	♂♂	♀♀	Total	Percent ♂♂
32	..	272	806	1078	25.2

Thus, it can be considered as established that if the male descends from the pure normal line, the progeny will always be normal whatever may be the genetical structure of the female. On the contrary, males, directly

TABLE 9

SERIES	NUMBER OF NORMAL CULTURES	NUMBER OF "FEMALE" CULTURES	NUMBER OF CULTURES WITH OFFSPRING LESS THAN 20 FLIES
♂ from No. 1828 × ♀ from normal line	10	10	7
♂ from No. 1851 × ♀ from normal line	12	13	7
Total	22	23	14

descending from line 3, give a "female" offspring even when mated with pure normal females. Hence the inevitable conclusion, that in the present case the character of the numerical sex-relations is entirely determined by the genetic structure of the father. Whether the offspring will be normal or "female" depends only on the male taken for mating; the female does not play any part here. This was confirmed afterwards by matings of numerous males to each of several different females; the results obtained showed once more that the sex-ratio of the offspring is determined by the structure of the father only.

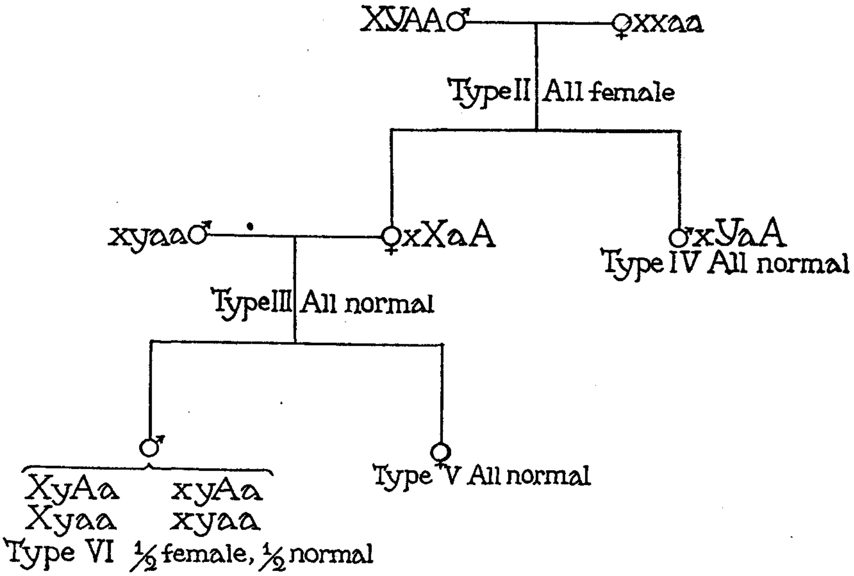

Figure 4

To explain the inheritance of the observed phenomenon by a violent alteration in the chromosomal apparatus, like non-disjunction, attachment of chromosomes, translocation or polyploidy, appears to be utterly impossible. Neither can plasmatic heredity explain the results of the crosses described. If we turn to the normal chromosomal mechanism, three hypotheses are possible. The factor, determining the abnormality studied, can be transmitted either by the Y or by the X-chromosome, or lastly by one of the autosomes. Figure 4 represents the inheritance of the sex chromosomes and the autosomes proceeding from line 3 (that is, those chromosomes capable of carrying the factor for the abnormality) throughout all the crosses described. For the sake of clearness, only one pair of autosomes is figured in the drawing. The chromosomes from line 3 are indicated by capitals.

It is easy to show that the Y-chromosome is not concerned in the transmission of this factor. The males that carry the Y-chromosome that comes directly out of line 3 nevertheless give a perfectly normal progeny (in type IV). On the other hand, males with a Y-chromosome originating from the pure normal line are capable of giving "female" cultures (type VI). This gives sufficient evidence that the Y-chromosome, whatever may be its origin, plays no part in the hereditary transmission of the gene calling forth the abnormality.

Examining the inheritance of the autosomes from line 3 we see that during the crosses described they could never attain a homozygous state. Consequently, if the factor which determines the appearance of the "female" ratios is transmitted by means of an autosome, it must be capable of acting in a heterozygous state; only thus could the appearance of abnormal sex relations in the crosses of type VI be explained. All the males from No. 1644 gave a "female" progeny; therefore they all carried the gene determining the abnormality. Even if we suppose them to have been only heterozygous for this gene, nevertheless at least half of their sons ought to have inherited it, in case it were really localized in one of the autosomes. However, their sons show in their offspring exclusively normal sex-ratios (in type IV). Therefore the supposition that the gene which causes the appearance of "female" culture lies in an autosome, must be rejected.

There remains the possibility of its being localized in the X-chromosome. For a verification of this, the males obtained from the cultures of type III must be examined. Half of these males carry the X-chromosome received from line 3; likewise, just the same quantity of males from type III have given "female" offspring. Were it possible to prove that the "female" culture were obtained exactly from those males, which carried the X from line 3, this would prove the correctness of the supposition that the gene determining the abnormality is transmitted by the X-chromosome.

The remaining males from the cultures of type III bear the X-chromosome proceeding from the normal line. These males, according to their genotypical structure, must be subdivided into two groups. The males of one group do not in any wise differ from the males of the normal line: all their chromosomes are perfectly normal. It is evident that this group could not yield "female" cultures. The males of the other group have only the autosomes originating from line 3. As was already proved, the autosomes do not contain the gene producing the abnormality; hence these males likewise cannot give an offspring with the "female" sex-ratio.

Thus, only such males can produce a "female" progeny, as derive the X-chromosome from line 3 of *D. obscura*, and this includes all of them, because they quantitatively exactly correspond to the "female" cultures of type VI: half the cultures of type VI gave a "female" offspring, and half of the males from type III carry an X-chromosome from line 3. In this way, the supposition that the gene which determines the capacity of males to produce a "female" progeny is really localized in the X-chromosome, can be considered as established.

ACTION OF THE GENE PRODUCING THE ABNORMALITY

Analysis by hybridization has led to the conclusion that the factor which calls forth the appearance of "female" cultures is transmitted by the X-chromosome as an ordinary sex-linked gene. Turning to the action of this gene we see that, first of all, the males carrying it produce a progeny with a numerically irregular relation of the sexes. On the contrary, the females which contain it, whether in a heterozygous or a homozygous state, nevertheless remain perfectly normal. This can be seen, for example, in the crosses of type III for the heterozygous females, and in the cross No. 1644 (and the like) for homozygous ones. In this way, the hereditary factor which causes the appearance of "female" cultures is manifested exclusively in the males; in other terms, it is not only sex-linked, but likewise sex-limited.

In order to penetrate somewhat deeper into the mechanism of its action, all the known cases of sex-ratio abnormalities are to be kept in view. The diversity of these deviations can be reduced to a comparatively small number of causes, which may be classified as follows:

I. Causes that influence the normal mechanism of distribution:

1. Parthenogenesis.
2. Unequal participation of different kinds of gametes in fertilization.

II. Causes exercising no influence on the normal mechanism of distribution:

1. Destruction of the zygotes of one sex through the injury of the gametes, which participate in fertilization.
2. Destruction of the zygotes of one sex, owing to their containing lethal factors.
3. Different resistance of the sexes to external influences.
4. A complete transformation of sex, as the extreme degree of intersexuality.

In order to decide to what group the phenomenon observed in *D. obscura* belongs, all the possibilities ought to be examined.

It is easy to establish at once, that the causes, enumerated for the first and the last categories (parthenogenesis and complete sex transformation) are totally inadequate to explain this abnormality. Parthenogenesis is entirely impossible, there being here a transmission of the X-chromosome from the father to its offspring (for instance, in the crossings of type II). The supposition that the observed abnormality is caused by a complete transformation of sex must also be rejected. Not once did I find in my cultures any individual bearing intersexual characters, although all the flies underwent a careful examination; neither did the anatomical dissection of numerous flies from the "female" cultures present any abnormalities. Further, a male of Drosophila, transformed into a female, must give two kinds of gametes—those with a Y and those with an X-chromosome. The combination with similar gametes of a normal male would present four types of zygotes: XX, 2XY and YY (the last perishes). Consequently, in the progeny of such transformed males a sex-ratio of 1 female to 2 males ought to be found—a thing that was never observed during the present work. Lastly, in some cases (for instance in the crosses of type II) the missing males are such as ought to be perfectly normal in regard to their genotypical structure; therefore, their transformation is of course highly improbable.

Passing on to other possibilities, it is necessary first of all to answer the question: Does one of the sexes perish in the process of development, or are both kinds of zygotes produced in different quantites from the very beginning? Happily, in Drosophila the problem can be solved. As is shown by the genetic and physiological experiments of other investigators, the female of Drosophila lays all the fertilized eggs, whether they be capable of development or not. By counting the number of eggs laid and then that of the flies developed out of them, one can determine the percent of the embryos that have perished and even the stage when their death took place. Such researches have been more than once carried out with *D. melanogaster* and the methods have been sufficiently well elaborated.

Table 10 gives results of analogous experiments undertaken to answer this question in *D. obscura*. It was necessary firstly to determine the percent of perishing embryos in the cultures of the pure normal line; the number obtained were to serve as a control. The results showed that nearly all the eggs began to develop, and only 2 percent of them perished. On the contrary, the percent of larvae that succumbed was rather large; this may be attributed to the unsatisfactory nature of the diet. The experiments were carried on in a thermostat with a constant temperature of 25°C. The methods used were the same as described in the recent work of J. CHI LI (1927). A second series of experiments, carried out under the same conditions, was organized with flies homozygous for the gene which causes the appearance of "female" cultures. The results obtained here turned out to be extremely like the data given by the control; the percent of eggs and larvae that perished was almost exactly the same as in the normal line.

TABLE 10

SERIES	NUMBER	EGGS	LARVAE	FLIES	EGGS DIED (percent)	LARVAE AND PUPAE DIED (percent)	PERCENT ♂♂
Normal line	1780	108	106	74	1.9	30.2	51.4
	1783	84	80	58	4.8	27.5	46.6
	1785	127	127	89	0.0	29.9	44.9
	1936	105	101	69	3.8	31.7	47.8
	Total	424	414	290	2.4	30.0	47.6
Line 3	1940	125	123	85	1.6	30.9	5.9
	1943	135	130	81	3.7	37.7	2.5
	1944	85	85	55	0.0	35.3	3.6
	1951	98	98	77	0.0	21.4	5.9
	1959	67	64	49	4.5	23.4	6.1
	Total	510	500	347	2.0	30.1	4.6

Thus, direct experiments, made in order to solve the question whether there exists in the "female" cultures of *D. obscura* a differential removal of one of the sexes in the course of development, give a clearly negative answer. Therefore it must be concluded that there is here a real alteration in the mechanism regulating the normal sex-destribution, that is, an alteration of the mechanism of sex-determination.

CONCLUSION

Analysis of the irregular sex-distribution in *D. obscura* shows that this phenomenon is due to the presence of a sex-linked gene. The action of this gene is analogous to that of a gametic lethal; the greater part of the spermatozoa determining the development of males do not participate in fertilization, consequently they are lost for the offspring. To say with greater precision on the strength of the results obtained, what constitutes the action of this gene, seems at present impossible, but nevertheless some suppositions on the subject can be made. Only two possibilities have to be reckoned with here: either the "female" progeny is determined by an unequal formation of spermatozoa with the X and Y-chromosomes during spermatogenesis; or else, even if the quantity of both kinds of spermatozoa formed be equal, the preponderance of females in the offspring can be explained by the inability of the sperms with the Y-chromosomes to compete with the sperms carrying the X. The last supposition seems more probable, as in this way the appearance of a certain number of males in the "female" cultures is easier to explain, but it is as yet impossible to decide finally to which of the two described types the observed phenomenon belongs. Anyhow, it can be considered as certain that the spermatozoa with the Y-chromosome participate in the fertilization process in but an insignificant number, while the spermatozoa carrying the X-chromosome (and therefore the gene that calls forth the abnormality) fecundate unhindered the great majority of eggs.

It is worth while to note that this gene was first obtained from nature. Out of 19 wild females which were caught two were heterozygous for it, thus giving us the right to suppose that it infects in a comparatively high degree the local *D. obscura* population. The gene is sex-linked, therefore it ought to respond to the influence of natural selection with particular directness, as no inbreeding is required for its manifestation. Hence the conclusion can be drawn that since it exists in the natural population it is probably useful, or at least harmless for the evolution of the given species. If this supposition be correct, the extension of this gene among the wild population must be expected even without the action of positive selection. Usually a sex-linked gene is transmitted by the father to a half of the descendants only, whereas here all or nearly all the flies receive it with the X-chromosome of the father; this favors its extension.

It is interesting to note that an apparently quite similar sex-ratio abnormality was found by STURTEVANT in *Drosophila affinis* (MORGAN, BRIDGES and STURTEVANT, 1925). He gives the following description of this case: "The data obtained indicate that a great deficiency of sons was obtained from certain males, regardless of the source of the females to which such males were mated. The few males obtained from such matings were, usually at least, normal in behavior; but some of the sons of the females from such anomalous cultures again gave very few sons." Unfortunately this interesting stock was lost before a more detailed analysis of it could be undertaken, so that it is impossible to determine whether the causes of both the abnormalities are the same. However, it seems very probable that the mechanism of both cases must be identical at least in regard to its essential features.

SUMMARY

1. The sex-ratio in the normal lines of *Drosophila obscura* is very near to the theoretical 1:1.

2. Out of 19 females caught in nature, two were heterozygous for a gene which causes strong deviations in the normal sex distribution.

3. The researches made have shown that this gene is localized in the X-chromosome and is transmitted like an ordinary sex-linked gene.

4. This gene is absolutely sex-limited, as it is not manifested either in heterozygous or homozygous females.

5. The males bearing this gene give in their progeny about 96 percent of females and only about 4 percent of males.

6. This gene has no influence on the development of the zygotes already formed, but acts directly upon the mechanism of sex-distribution. It provokes a sharp preponderance of females by almost totally removing the spermatozoa with the Y-chromosome from the fertilization process, acting thus like a gametic lethal (in the genetic sense of this term).

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