GEOGRAPHICAL DISTRIBUTION AND CYTOLOGY OF "SEX RATIO" IN DROSOPHILA PSEUDOOBSCURA AND RELATED SPECIES

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INTRODUCTION

A gene has been found in wild populations of several related species of Drosophila, which causes any male which carries it to produce few or no sons. The cytological analysis has shown why this is so, and also bears on several problems in chromosome behavior. The gene is widely distributed, both geographically and taxonomically, and raises a number of problems in population mechanics.

HISTORICAL

In 1922 one of us found (MORGAN, BRIDGES, STURTEVANT 1925) a strain of *Drosophila affinis* in which occasional males produced families consisting almost entirely of females. The data suggested that this peculiarity was due to the nature of the X chromosome carried by such a male, but the strain was lost before a detailed study could be carried out.

GERSHENSON (1928) later found a similar gene in the European *Drosophila obscura*, and made a careful analysis. He showed that the gene is in the X, and that a male carrying it produces very few sons, regardless of the nature of his mate. Females homozygous or heterozygous for the gene give normal (1:1) sex ratios when mated to normal males, and are fully fertile. Mated to males carrying the sex ratio gene they give the same excess of daughters as do normal females in these circumstances. Egg counts showed that the "sex ratio" result is not due to the death of male zygotes, since there is no greater mortality from such cultures than from controls giving a 1:1 sex ratio. The "sex ratio" gene thus has the effect of causing a male that carries it to produce nearly all X sperm, instead of the usual 50 percent.

GERSHENSON found the "sex ratio" gene to be present (heterozygous) in two of nineteen wild females studied. He pointed out that the gene should automatically increase in frequency in any population, since a male that receives it from only one parent transmits it to nearly all his offspring, while in females it is transmitted like any other gene. In fact such an increase does not occur, but the reason for this is unknown.

In 1929 this same gene, or at least a gene with the same properties, was found to be present in wild *Drosophila pseudoobscura* near Pasadena. The

TABLE 1

Tested X chromosomes in wild females (N—normal, S—sex ratio)

LOCALITY	YEAR	N	S
Race A			
British Columbia			
Shuswap Lake	1934	3	0
Arrowhead	1934	4	0
Nakusp	1934	5	0
Kaslo	1934	2	0
Washington			
Metaline Falls	1934	7	0
Idaho & Montana			
Lake Coeur d'Alene	1934	11	0
Bitterroot Mountains	1934	3	0
Oregon			
Days Creek	1933	2	0
Wyoming			
Big Horn Mountains	1935	3	0
South Dakota			
Black Hills	1935	4	0
Nebraska			
Scottsbluff	1935	4	0
California			
Mount Lassen	1934	3	0
Lake Tahoe	1935	2	0
Sequoia Nat. Park	1933	6	0
Santa Lucia Mountains	1934	49	1
Pasadena	1932	2	0
Pasadena	1933	5	3
Pasadena	1935	14	3
Fish Canyon	1930	5	3
Big Bear Lake	1932	4	1
Big Bear Lake	1934	12	0
Corona del Mar	1932	3	1
Henshaw Lake, near San Diego	1934	7	1
Nevada			
Charleston Peak	1935	3	2
Utah			
Cedar City	1934	2	0
Cedar City	1935	4	0
Zion Nat. Park	1934	2	2
Zion Nat. Park	1935	2	2
Bryce Nat. Park	1935	6	0
Northern Arizona			
Grand Canyon	1935	5	0
Flagstaff	1935	4	2
Southern Arizona			
Santa Catalina Mountains	1935	8	4
Santa Rita Mountains	1935	10	2
Chiricahua Mountains	1935	2	4
Race A			
Colorado (North)			
Aspen	1934	2	0
Estes Park	1935	5	0
University Camp	1935	4	1
Pikes Peak (slope)	1935	5	1
Pikes Peak (tree line)	1935	5	1
Colorado (South) & New Mexico (North)			
Mesa Verde Nat. Park	1935	5	1
San Juan Mountains	1935	6	0
Zuni Mountains	1935	4	2
Taos	1935	3	0
Southern New Mexico			
Pinos Altos Mountains	1935	4	2
Magdalena	1935	3	3
Carizozo	1935	4	2
Mexico			
Otinapa, Durango	1935	1	1
Cuernavaca, Morelos	1935	6	2
Cerro San Jose, Oaxaca	1935	4	0
Race B			
British Columbia			
Quesnel	1934	1	0
150-mile House	1934	1	0
Oregon			
Newport	1933	8	0
Reedsport	1933	13	1
Gold Beach	1933	11	3
Days Creek	1933	4	0
Oregon Caves	1933	4	2
California			
Klamath	1933	14	6
Eureka	1933	12	3
Shelter Cove (Humboldt)	1933	10	2
Mendocino	1933	4	0
Mount Lassen	1934	5	2
Lake Tahoe	1935	1	0
Sequoia Nat. Park	1933	7	3
Santa Lucia Mountains	1934	8	0

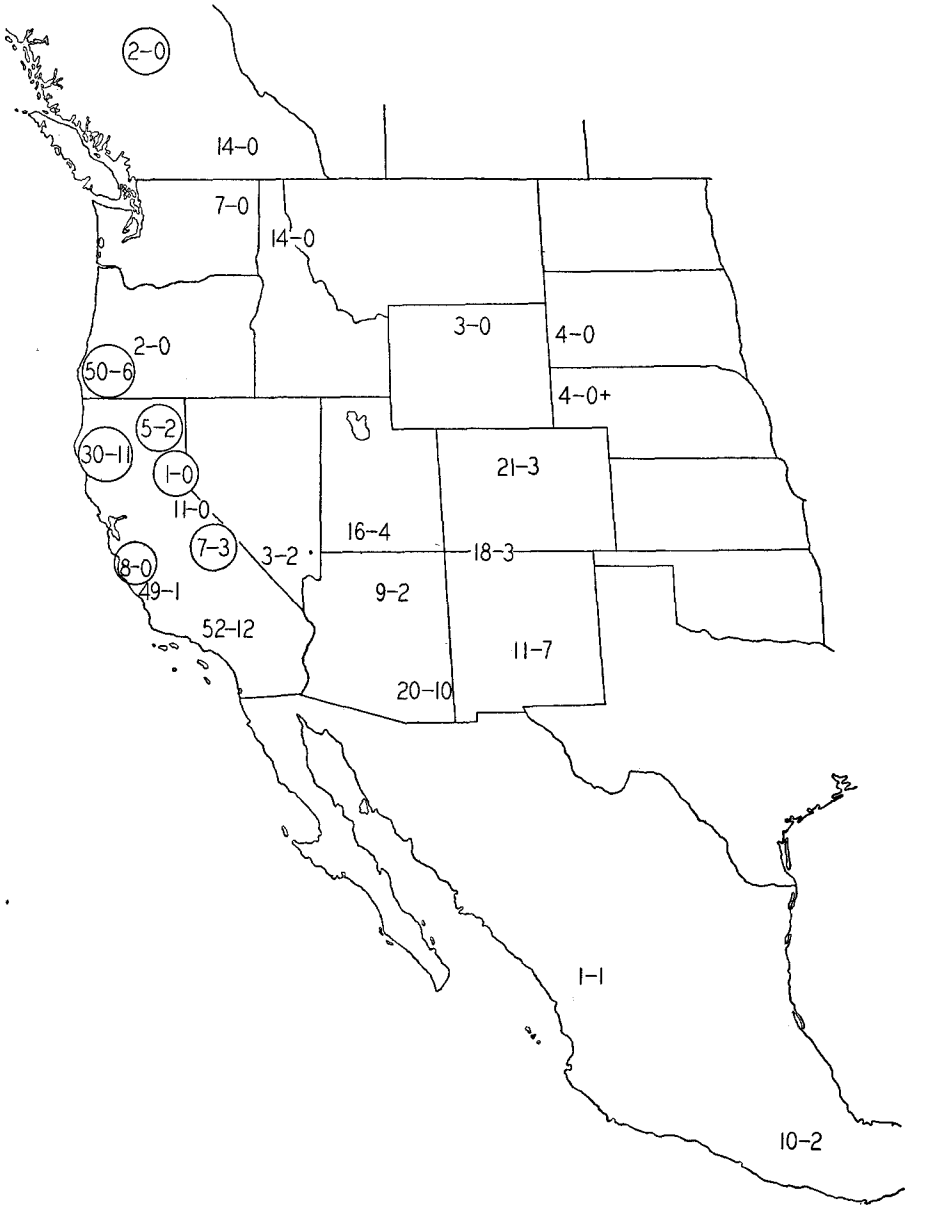


FIGURE 1. Geographical distribution of "sex ratio" factors. In each pair of figures the first indicates the number of tested X chromosomes in which no "sex ratio" was present, and the second shows the number of X's containing "sex ratio." Figures in circles—race B, figures without circles —race A.

present paper gives the results of our study of the geographical distribution and the cytological properties of this form, based chiefly on *D. pseudoobscura*. We have also made additional observations on *Drosophila affinis* and on two undescribed species near *D. affinis* in which "sex ratio" also occurs.

GEOGRAPHICAL DISTRIBUTION OF "SEX RATIO" IN DROSOPHILA PSEUDOOBSCURA

Tests soon showed that the "sex ratio" gene is present in both races (A and B) of *D. pseudoobscura*. Table 1 and figure 1 show its range. These data are based on tests of individual sons of females that were trapped out-of-doors. The attempt was made to test at least five sons from each female studied; in many cases fewer tests were actually made. The following convention was adopted: if both kinds of sons (normal and "sex ratio") were recovered, whatever the total number tested, it was considered that both X's of such a female were tested; if two were tested and were found to be alike it was considered that only one of her X's was tested; if three or more like sons were found it was assumed that both X's of the mother were tested. Other kinds of tests have shown the existence of sex ratio in a few other localities not shown in table 1: one of the wild females collected at Scottsbluff, Nebraska (Race A), and one of those at Days Creek, Oregon (Race B), had mated in nature with a sex ratio male and gave a typical "sex ratio" result. These, together with the records from wild males (table 2) have not been included in the map (figure 1), since they are not directly comparable quantitatively with table 1.

TABLE 2

Tested wild males (N—normal, S—sex ratio)

LOCALITY	YEAR	N	S	LOCALITY	YEAR	N	S
Race A				Santa Lucia Mts.	1934	45	2
California				*Utah*			
Big Bear Lake	1932	9	0	Bryce Nat. Park	1934	1	0
Big Bear Lake	1934	10	0	Cedar City	1934	3	0
Claremont	1932	2	0	Zion Nat. Park	1934	1	1
Fish Canyon	1930	3	1	*Nevada*			
Pasadena	1932	8	3	Las Vegas	1935	3	0
Arroyo Seco	1935	3	0	*Arizona*			
Henshaw Lake	1934	1	1	Gila, near Yuma	1935	0	1
Providence Mts.	1935	1	0	*Race B*			
Upper Kern Valley	1934	1	0	Upper Kern Valley, Calif.	1934	10	0

The map (fig. 1) indicates that the "sex ratio" gene has a wide distribution in both races. In the case of race A it has a maximum frequency near the Mexican border, and decreases to the north, disappearing not far

from the latitude of the northern boundary of California. The detailed data suggest also that the frequency decreases with increase in altitude. In southern California the Big Bear locality (7,000 feet) is much higher than the others (all under 4,000 feet), and has a lower frequency (1/17 as opposed to 11/47). Zion Canyon, Utah (where 4 out of 8 tested chromosomes carried sex ratio) is much lower than the neighboring localities (Cedar City, Bryce National Park, Grand Canyon) from which 17 tested chromosomes were all normal. In both of these cases the tests of wild males agree with those from sons of wild females.

The distribution of "sex ratio" in race B is less clear. There may be an area of maximum frequency on the coast near the Oregon-California line; but more data from other regions are needed.

PROPERTIES OF "SEX RATIO"

Table 3 shows the results obtained from a representative series of "sex ratio" males. It will be observed that the number of males per 100 females ranges from 0 to 17, with an average of about 3 in race A, 8 in race B. The exact numbers are probably not significant, since there are indications that the ratio may be modified by external agents such as tempera-

TABLE 3

Tests of "sex ratio" males (no sex-linked mutants present to produce viability complications)

RACE A				RACE B			
LOCALITY	♀♀	♂♂	♂♂ PER 100 ♀♀	LOCALITY	♀♀	♂♂	♂♂ PER 100 ♀♀
Wild males				*Sons of wild females*			
Zion Canyon	273	0	0	Gold Beach	873	66	8
Henshaw Lake	319	0	0	Oregon Caves	1063	29	3
Gila, near Yuma	303	2	1	Klamath	2584	262	10
Sons of wild females				Eureka	916	54	6
Pikes Peak	297	5	2	Shelter Cove	1334	110	8
Zuni	188	6	3	Lassen	657	74	11
Magdalena	304	4	1	Sequoia	529	58	11
Carizozo	116	20	17				
Pinos Altos	331	4	1				
Santa Catalina	374	26	7				
Flagstaff	460	0	0				
Chiricahua	528	5	1				
Santa Rita	379	6	2				
Zion Canyon	1101	10	1				
Santa Lucia	392	13	3				
Pasadena	239	9	4				
Durango	393	1	0				
Cuernavaca	743	0	0				

ture, and also that it may be influenced by modifying genes. As will be seen from the table, however, there is never any difficulty in determining whether a given male is giving the usual ratio of approximately 100 males per 100 females or the "sex ratio" result.

The sex ratios given by females carrying the gene in question (either homozygous or heterozygous) depend on the male used, and are not different from those given by homozygous normal females. It does not seem necessary to present numerical data to establish this relation.

Preliminary experiments soon established that the "sex ratio" gene is sex-linked, and attempts were then made to locate it with reference to the known sex-linked genes of *pseudoobscura*. Here an unexpected result was obtained: females heterozygous for "sex ratio" were found to give greatly reduced crossing over in the right limb of the X. This result has been obtained with "sex ratio" from several different localities (Santa Lucia, Pasadena, Chiricahua, Pikes Peak, Pinos Altos, Magdalena, Durango in race A; Santa Lucia, Sequoia in race B), and it has been shown (by testing crossovers) that the reduction in crossing over is definitely associated with

TABLE 4

Frequency of crossing over in various crosses involving the standard and the "sex ratio" in race A and B. In each case the frequency of crossing over is shown in percentages, and below is shown the total number of flies on which this percentage is based.

LOCI / CROSS	*sc-y*	*sc-w*	*sc-f*	*y-v*	*y-co*	*w-s*	*v-f*	*v-s*	*f-mg*	*f-s*	*mg-s*	*co-s*
A+/A+				12.6			4.0		20.0		47.0	
				3617			1616		643		251	
A+/A *sr*				13.3			2.1		4.5		0.5	
				1732			1571		920		920	
A+/B+	0.0			8.7	23.8			25.4				4.7
	109			492	235			492				235
A+/B *sr*	0.1				28.2							48.2
	882				882							882
A *sr*/A *sr*				16.5				43.2				
				176				176				
A *sr*/B+		7.4				6.8						
		68				216						
B+/B+			10.0							47.1		
			1109							1109		
B+/B *sr*			13.7							4.4		
			1170							1170		

"sex ratio." Genetic studies of homozygous Pasadena "sex ratio" and cytological studies (in cells of the larval salivary glands) of Santa Lucia and Zion "sex ratio" heterozygotes agree in demonstrating that this reduction is due to the association of "sex ratio" with an inversion of a section of the right limb of the X chromosome, lying not far from the free end of the chromosome. The length of the inversion is equal to approximately one-fifth of the length of this limb, as observed in the salivary gland chromosomes. The only other crossover reducer met with in this chromosome limb genetically, and the only other inversion seen in it cytologically, is that in which races A and B differ from each other (LANCEFIELD 1929, TAN 1935). Accordingly it was desirable to see if race B "sex ratio" is also associated with an inversion, and also how the *sr* sequence of race A is related to the normal race B sequence. This problem has not been studied cytologically; but the genetic data of table 4 show the answer. There are three different sequences: the standard A which is identical with *sr* B; *sr* A; and standard B.

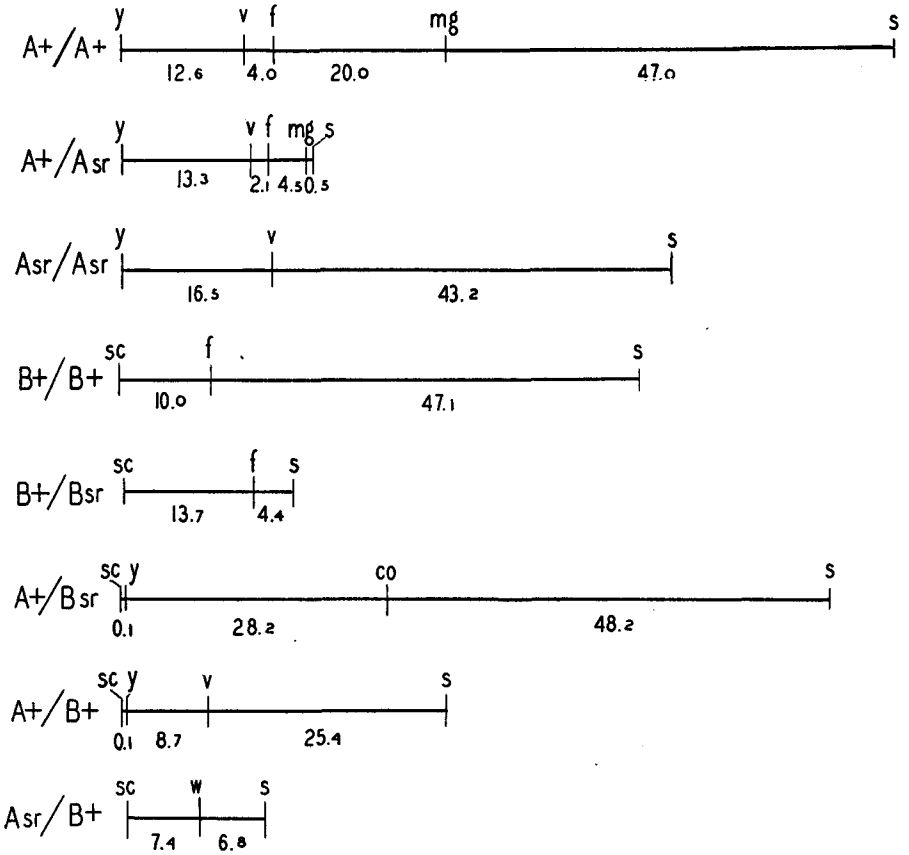


FIGURE 2. Crossing-over frequencies in the X chromosomes in various crosses.

In the case of standard A/*sr* B the actual data obtained are shown in table 5. Contrary classes are not equal in this case, a circumstance that is usual in A-B crosses (DOBZHANSKY and STURTEVANT 1935). However, it is clear that crossovers in both regions 2 and 3 (the latter including the "sex ratio" gene) are viable. None of the classes showed unusual characteristics in excess of the high variability usual in the offspring of A-B backcrosses, so it may safely be assumed that no net duplications or deficiencies were produced, that is, the sequence of genes in the two chromosomes was identical. In general the A-B hybrids show rather more crossing over than is to be expected by comparison with pure A or pure B. This is due to the effects of inversions in other chromosome limbs (SCHULTZ and REDFIELD, in MORGAN, BRIDGES, and SCHULTZ 1932).

TABLE 5

Crossing-over in (A+) / (B *sr*) *sc*	*y* (1)	*co* (2)	*s* (3)
(+^{sc} classes in left-hand column; male offspring only)			
Non-crossovers	81	245	
Singles, Interval 1	1	0	
Singles, Interval 2	93	37	
Singles, Interval 3	122	184	
Doubles, 2 and 3	65	54	

TESTS WITH II-Y TRANSLOCATION

SCHULTZ (1933) has reported briefly on a dominant mutant type known as Delta, that was associated with a II-Y translocation. Delta males, mated by normal females, gave mostly normal daughters and Delta sons, plus a few Delta daughters (due to the production of XY sperm) and a few normal sons (XO, due to the production of sperm with neither X nor Y). This translocation was tried against "sex ratio" (Pasadena, A). From normal ♀ × *sr*/Delta ♂ there were produced 1907+ ♀, 166+ ♂, 14 Delta ♂. This result was unexpected, and remained unexplained until the cytological study described below. It is now clear that it was correct in indicating that the Y chromosome is actually absent in most of the sperm of a "sex ratio" male. In the present case the absence of Delta females is even more marked than in the case of +/Delta male, where about 2.6 percent of the daughters were Delta; by comparison at least 49 should have been expected here, whereas none was found.

SPERMATOGENESIS IN "SEX RATIO" MALES

As shown above, the offspring of a male carrying "sex ratio" come from eggs fertilized by spermatozoa possessing an X chromosome but no Y

chromosome. Only a small fraction of the eggs are fertilized by Y-bearing sperm, or by spermatozoa free from either X or Y chromosomes. Since no significant increase of zygotic mortality is observed in sex ratio cultures, the possibility that the eggs fertilized by Y-bearing spermatozoa are inviable is excluded. Hence, the explanation of the behavior of the "sex ratio" may be sought along one of the following two lines. First, the X-bearing and the Y-bearing spermatozoa may be produced in equal numbers, but a majority of the latter may for some reason fail to fertilize the eggs. Second, the spermatogenesis may be so modified that only X-bearing sperm are produced. A cytological study was undertaken with the aim of securing some evidence bearing on this problem. A strain of race A rather than of race B, was selected for this purpose, because in the former the X and Y chromosomes are clearly distinct from each other in meiotic stages, whereas in race B the two chromosomes are more similar.

FIGURE 3. Spermatogonial metaphase plates from males carrying "sex ratio." X=X chromosome, Y=Y chromosome.

Males from one of the "sex ratio" strains were crossed to females homozygous for the sex-linked recessives eosin, magenta, and short. The F_1 females from cultures showing abnormal sex ratios were crossed to normal males, and in the next generation non-magenta males were selected. Since "sex ratio" is closely linked with magenta, these males must carry it. Testes of young males were fixed in Benda's or Navashin's fluid, sectioned 7μ thick, and stained in iron haematoxylin. In the following description the spermatogenesis of the "sex ratio" males is compared with that in normal ones. For the information concerning the latter see the papers by DARLINGTON (1934) and DOBZHANSKY (1934).

The size, shape, and general structure of the testis in "sex ratio" males is normal. The spermatogonia are likewise normal; their resting nuclei have one, less frequently two, nucleoli with one or two satellites. The spermatogonial divisions (fig. 3) show a V-shaped X and a J-shaped Y chromosome, the somatic pairing being apparently as strong as in normal spermatogonia.

The first spermatocytes (fig. 4a) have nuclei with the usual reticulum and several nucleoli of various sizes. Especially in preparations fixed in Navashin's fluid, a careful examination shows that one of these nucleoli,

usually the largest one, is really a precociously condensed chromosome. It is split equationally, and appears as a very short and stout V, or as a broken ring, or as an angular mass. In strongly destained preparations this chromatin nucleolus, which is the Y chromosome, retains the stain longer than other nucleoli (fig. 4a). Since a precocious condensation of the Y chromosome has not been noticed in the normal spermatogenesis, a reexamination of the slides of normal testes has been made. The normal first spermatocytes have from one to several nucleoli; the single, or the largest of the several, appears usually as a regular sphere. In strongly destained or faded preparations the sphere shows a lighter core and a dark

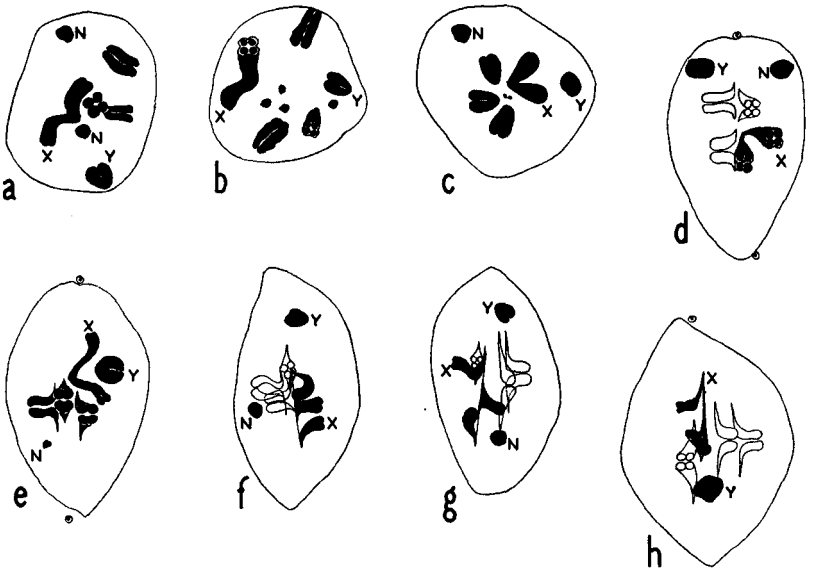


FIGURE 4. Spermatogenesis in "sex ratio" males. a, b—diakinesis; c–h—metaphase and anaphase of the first meiotic division. N—nucleolar fragments; X—X chromosome; Y—Y chromosome. In d, f, g, and h only outlines of the autosomal bivalents are represented.

surface. Only seldom faint indications of doubleness in the nucleolus are noticeable. The relations between this nucleolus and the Y chromosome are uncertain. (Dr. HANS BAUER kindly informs us that in his preparations of the normal testes stained by the Feulgen method the nuclei of the first spermatocytes show no chromatin nucleolus. No satisfactory preparations of "sex ratio" testes stained in Feulgen's have been seen, but here the relation between the nucleolus and the Y chromosome is rather clear. It follows that the precocious condensation of the Y chromosome in the nuclei of the first spermatocytes is the first visible sign of abnormality in the spermatogenesis of "sex ratio" males.)

At diakinesis (figs. 4d, 5a, b) three autosomal bivalents and unpaired X and Y chromosomes become visible. The autosomal bivalents appear in

side view as two rather intimately paired rods, and in end view as four equidistant dots. The appearance and the behavior of the autosomal bivalents at diakinesis and the first meiotic division are normal. The Y chromosome is strongly contracted, but the equational split is usually clearly visible (Y, figs. 5a, b). In early diakinesis (fig. 4d) the autosomes and the X chromosome appear, as usual, first as pale brownish bodies which acquire the full stainability only gradually. The Y chromosome stains much darker; its origin from the "chromatin nucleolus" of the preceding stage can be followed with certainty. The X chromosome, despite

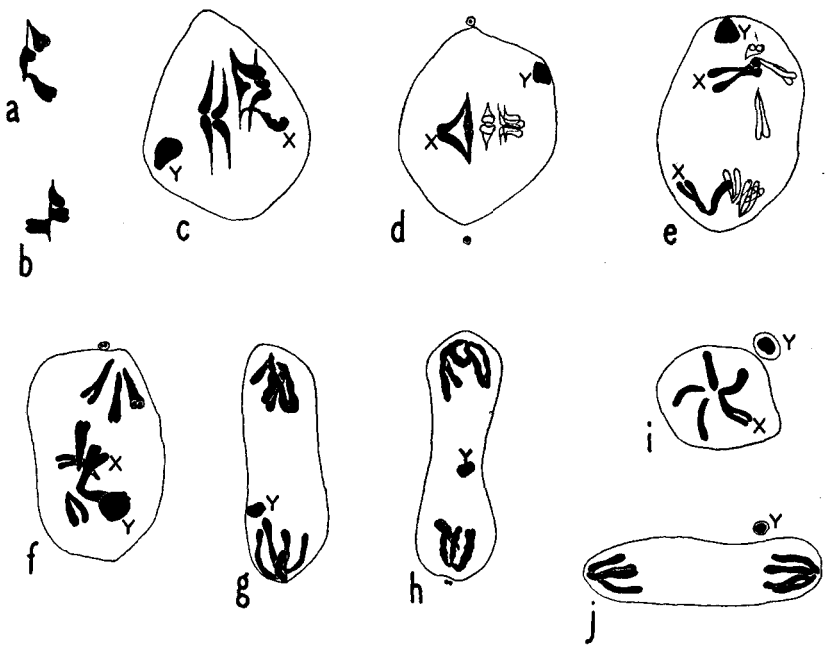


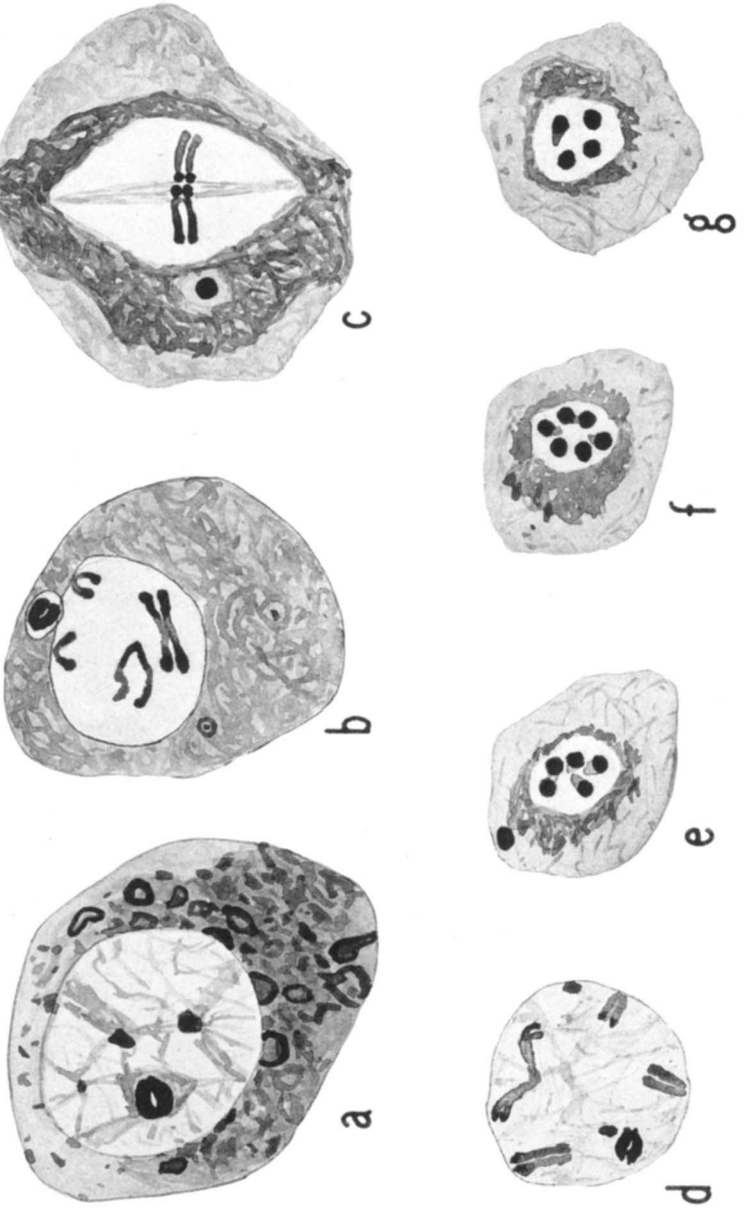
FIGURE 5. Spermatogenesis in "sex ratio" males. a and b—the X chromosome at anaphase; c and d—anaphase of the first meiotic division; e–h—telophase of the same; i—metaphase, and j—telophase of the second meiotic division. X and Y— the X the Y chromosomes respectively.

being an apparent univalent, is uncommonly large and stout (figs. 5a–d). In favorable cells, in which one or both ends of the X chromosome are directed toward the observer, the cause of this is visible: this chromosome has two equational splits instead of the normal one, and is therefore quadripartite instead of bipartite. Whether this quadripartite structure is present in all cells is doubtful, since in some the X chromosome is rather slender (figs. 4d, 5e), but it may be taken for certain that most cells do have quadripartite X's. It is, however, impossible to determine by direct observation how far the double equational split goes in the body of the chromosome (as just stated, it is visible in the ends only). Since in normal

gametogenesis each chromosome is split into two sister strands along its entire length except at the spindle attachment where the two strands are held together, it seems simplest to assume that here too we have four chromatids converging to an undivided spindle attachment. Such an interpretation is in agreement with the observations on the later stages.

At metaphase the autosomes and the X chromosome become arranged in the loose equatorial plate characteristic for the first meiotic division in *Drosophila pseudoobscura* (figs. 5c–h). The Y chromosome takes no part in the equatorial plate and may lie anywhere on the spindle, in the equatorial region as well as in the polar ones. The extraordinary persistence of the nucleolar fragments (N, figs. 5a–g) during the first division seems to be characteristic for the spermatogenesis in the "sex ratio" males. In normal spermatogenesis the nucleolus of the first spermatocytes becomes pale and disintegrates already during the mid-diakinesis, although in some cases the fragments are visible up to metaphase. In the "sex ratio" the fragments are seen even in early anaphase (figs. 5f, g), and some of them are so large and heavily staining that for a time it was suspected that the univalent Y chromosome divides in some cells as early as the first division, which proved to be an error.

At anaphase (fig. 4f–h, fig. 5a–c) the spindle attachment in the quadripartite X chromosome evidently undergoes a division, for the two daughter chromosomes, each showing one equational split, become directed toward opposite poles and away from each other. However, no immediate and complete separation takes place. One of the two arms of each daughter chromosome becomes free, while the other arms are held together for some time. The resulting configurations have a striking resemblance to those observed in the X-Y bivalents of normal *Drosophila pseudoobscura* by DARLINGTON (1934, fig. 20), and attributed by him to the presence of two reciprocal chiasmata in one of the two arms of each chromosome. Whether or not the association of the two X chromosomes in our case is also due to chiasma formation is uncertain. An alternative explanation would be that the second equational split takes place in one arm somewhat later than in the other, and that the unsplit arms are temporarily held together by some force, for instance by an exaggerated somatic pairing. This involves the assumption that the division of chromonemata occurs in a condensed metaphase chromosome. One must also take into account that in some cells, more or less exceptional ones, somewhat different configurations may be seen, as in figure 5d. Here both arms are associated with their homologues at their ends; one of the arms seems single rather than double, although this is not certain. In a few cells (fig. 5f) the division of the spindle attachment does not occur until late anaphase, and the quadripartite X chromosome lags on the spindle.


a
b
c
d
e
f
g

At late anaphase and early telophase (fig. 5e, g, h) three autosomal diads and one X chromosome may be seen passing to each pole. The X is split once equationally, and resembles in all particulars the X seen at one of the two poles in normal spermatocytes. The Y still continues to lag in the spindle; it remains much condensed and its spindle attachment end is not attenuated. The next stage is the division of the cell and the formation of second spermatocytes.

The second spermatocytes (Plate 1b) are normal except for the fact that in some of them besides the normal nucleus also a small micronucleus is found. The micronucleus contains a single chromosome which is evidently the Y chromosome. It follows that during the first meiotic division the Y is not included in either of the two telophase groups, but is left behind, and at the time of the fission of the cell body gets into one of the daughter cells forming there a separate small nucleus. The chromosomes in the second spermatocytes can frequently be counted; all among the sixty-four spermatocytes examined had an X chromosome and no Y chromosomes in their main nuclei. It should be remembered, however, that the "sex ratio" strain used by us for the cytological investigation produces practically only females, and that other strains give rise to some males as well. What happens in the latter is unknown; it is possible that cells like that shown in fig. 4e give rise to one second spermatocyte containing the X and one containing the Y. It is also possible that normal X-Y bivalents occur at the first division in some cells in "sex ratio" males. Some Y-bearing spermatids were found in our strain as well (see below).

The second meiotic division (Plate 1c, figs. 5i, j) is perfectly normal. The chromosomes in the main nucleus form an equatorial plate, and at anaphase the equational halves pass to the opposite poles. The micronuclei containing the Y chromosome take no part in this division. They are not included in the spindle, nor do they form small spindles of their own. The chromosome in the micronucleus becomes small and exceedingly contracted; the equational split in it is no longer visible. It is included in one of the resulting spermatids. At the telophase of the second division the chromosomes in the polar groups can be counted if the spindle is viewed from the pole (Plate 1c–g). Most cells show five dots (Plate 1e), and by focusing it is possible to see that two of the five dots unite at a lower level. These are the two limbs of the X, hence one X and three autosomes are present in such a telophase group. Such counts were made in 200 telo-

DESCRIPTION OF PLATE I

Spermatogenesis in "sex ratio" males. a—first spermatocyte; b—a second spermatocyte with a micronucleus; c—the second meiotic division; d—early diakinesis; e, f, and g—polar views of the telophase groups at the end of the second meiotic division.

phase groups. The results were: 195 groups with X but no Y; 3 groups with a Y but no X (Plate 1g); and 2 groups with one X and one Y (Plate 1 4f.)[1]

The spermatogenesis is normal: some of the young spermatids have a micronucleus with the Y chromosome, but in somewhat later stages the micronuclei are no longer visible. What is their final fate is uncertain; probably they are discarded together with the excess cytoplasm. Cross-sections of the cysts of mature or semi-mature spermatozoa offer the opportunity to count the number of spermatozoa per cyst. The normal number is close to 128 (meiosis occurs in groups of 32 cells, this results in 128 spermatids, DOBZHANSKY 1934). In the "sex ratio" males about four cysts were counted, and the resulting numbers were not far from 128. There is no indication whatever that any spermatids die, or that some of the spermatozoa in a cyst are abnormal.

OCCURRENCE OF "SEX RATIO" IN OTHER SPECIES

As pointed out in the introduction to this paper, "sex ratio" has been reported in *D. affinis* Sturtevant and *D. obscura* Fallén. These two species are rather similar to *D. pseudoobscura* Frolowa; there are also several European and North American species of the same group (mostly undescribed), some of which we have investigated. The European species are being studied by Mr. J. E. COLLIN (Newmarket, England), whose manuscript name *subobscura* is here used for the commonest British species. This is the form whose chromosomes are listed (*fide* C. W. METZ) by MORGAN, BRIDGES and STURTEVANT (1925, p. 182), under the designation "undescribed European species near *D. obscura*." The species here listed as *obscura* Fallén may or may not be the same as that used by GERSHENSON (1928), since the cytological account of FROLOWA and ASTAUROW (1929) indicates that at least two Russian forms occur, both different from METZ'S account of *subobscura*. We have in preparation an account of some of the American forms; the species here listed as *athabasca*, *azteca*, and *algonquin* will be described there.

Table 6 shows the data obtained from wild specimens of these various species—data of the same type as those of tables 1 and 2 show for *D. pseudoobscura*.

In the cases of *affinis* and *athabasca* from Woods Hole, and of *azteca* from Cerro San Jose, Oaxaca, Mexico, wild females were found that gave few or no sons. We may assume that these had mated with "sex ratio" males.

"Sex ratio" thus occurs in *pseudoobscura* A, *pseudoobscura* B, *obscura*, *affinis*, *athabasca*, and *azteca;* it was not found in *subobscura* or *algonquin*,

[1] This figure may also be interpreted as resulting from non-disjunction of one of the autosomes; if so an X, four autosomes, and no Y are visible in this group.

but may well be present in them in other localities. The question arises, are we dealing with the same gene in all these cases? That the essential properties are the same is clearly indicated. As shown above, in both races of *pseudoobscura* the gene is located in the right limb of X, is associated with an inversion, and males carrying it give similar frequencies of sons. In *obscura*, GERSHENSON has established all these points except the region occupied in the X and the presence of an inversion. In *affinis* we have found a similar frequency of sons, have shown the gene to be in the X, and salivary gland preparations of heterozygous females show an inversion in the X. In *athabasca* and *azteca* the evidence is less complete, resting chiefly on the sex-ratio itself, though some experiments with *athabasca* are at least consistent with the sex-linkage of the gene concerned.

TABLE 6

Occurrence of "sex ratio" genes in species other than D. pseudoobscura

SPECIES	LOCALITY	YEAR	X'S OF WILD ♀♀		WILD ♂♂	
			+	*sr*	+	*sr*
obscura	Berlin, Germany	1933	2	0	1	0
obscura	Birmingham, England	1932	2	0	3	0
obscura	Newcastle, England	1933	0	0	5	0
obscura	*Moscow, Russia (GERSHENSON 1925)	1925	36	2	0	0
subobscura	Berlin, Germany	1933	0	0	3	0
subobscura	Birmingham, England	1932	17	0	77	0
subobscura	Newcastle, England	1933	19	0	26	0
affinis	Woods Hole, Mass.	1932	4	0	12	1
affinis	Woods Hole, Mass.	1933	3	0	0	0
affinis	Woods Hole, Mass.	1935	12	2	95	8
affinis	Kushla, Ala.	1935	7	0	8	0
algonquin	Woods Hole, Mass.	1932	1	0	1	0
algonquin	Woods Hole, Mass.	1933	2	0	0	0
algonquin	Woods Hole, Mass.	1935	8	0	10	0
algonquin	Mendham, N. J.	1932	2	0	0	0
athabasca	*Woods Hole, Mass.	1935	6	1	1	2
athabasca	Quesnel, B. C.	1934	3	0	0	0
athabasca	Kaslo, B. C.	1934	4	0	0	0
athabasca	Shuswap Lake, B. C.	1934	2	0	0	0
athabasca	Arrowhead, B. C.	1934	2	0	0	0
athabasca	Cape Flattery, Wash.	1934	2	0	0	0
azteca	Cuernavaca, Mexico	1935	5	0	0	0
azteca	Oaxaca, Mexico	1935	2	0	0	0

* Species identification somewhat uncertain.

DISCUSSION

Some items in the spermatogenesis of the "sex ratio" males have a rather general interest. In the first spermatocytes the X chromosome undergoes two equational splits while the rest of the chromosomes split once. This shows that the division of the chromonemata is not necessarily induced by the physiological condition of the nucleus as a whole, but can proceed independently in different chromosomes. This is not new, for in some interspecific hybrids the univalent chromosomes may undergo splitting both at the first and at the second meiotic divisions, while the bivalents split equationally only once. Nevertheless, our case remains unique since here the extra split in the chromosome is known to depend upon the presence of a factor (or a group of factors) localized in a relatively short section of the chromosome involved. The further behavior of the quadripartite X chromosome, and especially the formation of the chiasma-like association between the division products in this chromosome, clearly has a bearing on a number of theoretical problems connected with crossing-over and general chromosome structure. Since, however, we do not consider it established that real chiasmata are here found, a further discussion of this point is premature.

Following DARLINGTON, one might suppose that the reason for nonpairing of the X and Y chromosomes in the "sex ratio" gametogenesis is the extra split in the X chromosome. This, however, helps little in accounting for the subsequent behavior of the X and Y chromosomes. Why, for instance, does the Y chromosome show a precocious condensation and heteropycnosis, and the X behave like the autosomes? Or why is the Y chromosome left in the cytoplasm instead of becoming included in one of the telophase groups? Its univalent condition does not account for either of these phenomena, since the univalents in the hybrids between the A and B races of *Drosophila pseudoobscura* are frequently included in the telophase groups, and if they form separate micronuclei they show signs of activity at the next division (DOBZHANSKY 1934). The only visible difference between the univalents in the hybrids and the univalent Y in the "sex ratio" is that in the former the spindle attachment becomes active after a more or less prolonged delay (as shown by the attenuation of the attachment region), while in the latter no such "activity" is observed. The inherent difficulty of the causal analysis of the behavior of the Y chromosome in the "sex ratio" males lies in the fact that the genetic factor responsible for the whole complex of the deviations in the course of the spermatogenesis is located in the X chromosome only. Despite this, much of the abnormality concerns not the X but the Y chromosome.

"Sex ratio" is associated with an inversion (as compared with the normal X) in both races of *pseudoobscura* and in *affinis* (*obscura*, *athabasca*, and

azteca have not been examined for this point). It seems clear that this association cannot be an accidental one, but there are two reasons for concluding that the inversion as such is not an essential part of the mechanism responsible for the peculiarities of the "sex ratio" X. First, these peculiarities occur only in males which have a single X and are not heterozygous for an inversion, since the region of the X concerned is presumably not one that is in any sense homologous to the Y. Second and more significant, the "sex ratio" sequence of *pseudoobscura* race B is identical with the normal sequence of race A, so that "position effects" resulting from the inversion are excluded. One possible interpretation of the significance of the inversions is suggested below.

GERSHENSON (1928) pointed out that "sex ratio" should automatically increase in frequency in any population containing it, since a heterozygous male transmits the gene to nearly all his offspring, while a female (either homozygous or heterozygous) transmits it in the same frequency as any other gene. Clearly this would be fatal to the race if it did occur; we have, for example, collected a fertilized wild female that gave 236 daughters and one sterile son. It is equally clear that the expected increase in frequency does not occur in nature. Wild populations are somehow kept in equilibrium; but the nature of the counteracting influence can only be surmised. It must be of such a magnitude that it brings about a result equivalent to the production of only about half as many offspring by a "sex ratio" male as by a normal one, on the average. Such an influence should be easy to detect experimentally, but preliminary attempts to locate it have not been successful. These studies are being continued.

GERSHENSON'S point suggests a possible interpretation of the significance of the inversions associated with "sex ratio." If "sex ratio" is in reality not one gene, but two or more complementary ones located in the same general region of the X, then in the absence of an inversion the two would constantly be separated by crossing over and the automatic increase in frequency would be slow. If an inversion occurred in an X that happened to carry both of these hypothetical genes, their separation would be prevented and automatic increase would set in.

The algebraic analysis of populations containing "sex ratio" is difficult, and may best be postponed until more evidence is available concerning the nature of the equilibrium that occurs. These remarks apply even more obviously to the hypothesis of complementary genes just suggested.

SUMMARY

1. "Sex ratio" (symbol *sr*) lies in the right limb of the X of races A and B of *D. pseudoobscura*.

2. Males carrying *sr* give offspring consisting mostly of females, regardless of the nature of their mates.

3. Cytological study shows that, in "sex ratio" males, the X undergoes an equational division at each meiotic division, the Y degenerates, and the autosomes behave normally.

4. The *sr* gene is widely distributed in wild populations of both races of *pseudoobscura*, and what appears to be the same gene occurs also in wild populations of the closely related species *obscura*, *affinis*, *athabasca*, and *azteca*.

5. "Sex ratio" is associated with an inversion in both races of *pseudoobscura* and in *affinis*, the other three species not having been studied.

6. In *pseudoobscura* three sequences occur: *sr* A, normal B, and one that is common to normal A and *sr* B.

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