STUDIES ON HYBRID STERILITY. II. LOCALIZATION OF STERILITY FACTORS IN DROSOPHILA PSEUDOOBSCURA HYBRIDS

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THE PROBLEM

THE causation of hybrid sterility has long been one of the unsolved problems of biology. To date, probably the greatest advance in this field has been made by FEDERLEY who discovered a failure of the meiotic chromosome pairing in the sterile hybrids between moths of the genus Pygaera. This finding has since been amply corroborated by other investigators in sterile hybrids both in animals and in plants. Naturally enough it became tempting to suppose that the failure of the meiotic pairing is the cause of hybrid sterility. The restitution of the normal meiotic pairing as well as of fertility following the reduplication of the chromosome complement in allotetraploid hybrids seems to be further evidence in favor of this view. And yet, this view proves to be inadequate as a general explanation of hybrid sterility. Two difficulties deserve particular attention. First, some hybrids are sterile despite the fact that the meiotic pairing in their gametogenesis is apparently normal (MICHAELIS 1933 in Epilobium, DOBZHANSKY 1934 in some crosses in *Drosophila pseudoobscura*); while in other sterile hybrids the gametogenesis does not reach the meiotic stages (KERKIS 1933, in *Drosophila melanogaster* × *D. simulans*). Second, the failure of the meiotic pairing in sterile hybrids is usually attributed to an "incompatibility" of the chromosomes of species or races entering the cross. This, clearly, is a restatement of facts and not a causal explanation. It remains possible that suppression of meiotic pairing may be caused by different mechanisms in different cases, and that sometimes there is no cause and effect relation between the failure of pairing and the sterility.

In my previous publications (DOBZHANSKY 1933, 1934) a hypothesis was suggested according to which there exist at least two different types of hybrid sterility—the chromosomal type and the genic type. The chromosomal type is caused by differences in the gross structure (gene alignment) of the chromosomes of the parental forms, preventing, through competition in pairing, the normal conjugation of the chromosomes at meiosis, and causing their irregular disjunction. The genic type of sterility is due to interactions between complementary genetic factors contributed by both parents. If the genetic constitution of one of the parental forms is *SStt*, and of the other *ssTT*, the hybrid is *SsTt*. The assumption is made that the presence of the factor (or the group of factors) *S* alone, or of the

factor *T* alone, permits unlimited fertility, but that the factors *S* and *T* interact in such a manner as to make sterile an organism carrying them simultaneously.

Although some indirect evidence for the existence of these two sterility producing mechanisms is available (cf. DOBZHANSKY 1934), the above hypothesis cannot yet be considered proved. To date, in no case has a sufficient amount of gross structural differences between chromosomes of related species been demonstrated, nor have the theoretically postulated sterility genes been isolated and localized. The experiments to be reported in the present article are aimed at securing some information on this subject. The sterility of the male hybrids between race A and race B of *Drosophila pseudoobscura* is apparently of the genic type, (DOBZHANSKY and BOCHE 1933, DOBZHANSKY 1933, 1934). The results of LANCEFIELD (1929) and of KOLLER (1932) suggest that some of the differences producing sterility between these races are located in the X-chromosome, while autosomes seem also to be involved (DOBZHANSKY 1933a). Further attempts to localize the sterility genes in the chromosomes of *Drosophila pseudoobscura* are described below.

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METHOD

Factors whose interaction is responsible for the sterility of a hybrid are a part of the germ plasm, and hence must have a physical carrier in the gametes. The task is to find out in which of the constituents of the gametes these factors are localized. Races A and B of *D. pseudoobscura* produce in F_1 completely sterile male hybrids, but fertile females. The latter may be back crossed to males of either parental race. In the offspring of the backcrosses various combinations of the chromosomes and of the cytoplasms of the ancestral forms should occur in separate individuals. Some of the individuals may be expected to be sterile, and others fertile. Provided the chromosomes are marked by appropriate mutant genes, the genetic structure of a given individual may be recognized by its phenotype. Hence, it will be possible to determine which combinations of the ancestral elements are necessary to induce sterility, and which permit the individual to be fertile.

In practice, two experimental procedures are possible. First, by repeatedly backcrossing hybrid females to males of the same race, one may secure individuals carrying one of the elements (e. g., a chromosome or a

part thereof, or the cytoplasm) of one race, and all other elements from the other race. Second, among the male offspring of the first backcross generation individuals should be found representing many of the possible combinations of the above elements. Both experimental procedures should, probably, be made use of before a final solution of the problem here under consideration may be consummated. The data reported in the present article are based on the results of the application of the second procedure. The conclusions arrived at are, however, in accord with those suggested by the outcome of the few experiments of Doctor A. H. STURTEVANT and the writer involving the application of the first procedure.

Males appearing in the progeny of the backcrosses have testes ranging in size from normal (i.e., that found in males of either parental race) to very small. Males having small testes are invariably sterile, those with testes of normal or nearly normal size are mostly fertile. It has been shown cytologically (DOBZHANSKY 1933, 1934) that the disturbances of the spermatogenesis leading to sterility are greatest in the very small testes, and least in large ones. Hence, size of the testis is a fair measure of the degree of departure from the normal structure and functioning of the testis. This fact is very important for our purposes, since it permits a sort of quantitative expression of the results of investigation of the hybrid males. The smaller the testes in males of a given genetic constitution, the more (or the stronger) sterility factors they carry.

TECHNIQUE

Testis size in *D. pseudoobscura*, particularly in the hybrid males, is exceedingly sensitive to environmental factors. Although our conclusions are to be based on a comparison of testis size in different classes of males developed in the same culture bottles, and hence under identical environment, care was taken to insure homogeneous culture conditions in all experiments. Three to four F_1 hybrid females were placed in the same bottle with five to six males. Parents were kept in vials with food for four days, transferred (without etherization) to standard culture bottles, placed in incubator at 24.5° C., and allowed to oviposit for about four days, transferred to fresh culture bottles, and again left there for a similar length of time.

When the progeny of the backcross started to emerge, flies were classified according to the marking genes they carried, and males were dissected in physiological salt solution. Testes were isolated, and their length measured with the aid of an eyepiece micrometer (1 unit = 17.4μ). The technique of the measurements has been described by DOBZHANSKY and BOCHE (1933). The testis of *D. pseudoobscura* is ellipsoidal in shape; its greater diameter shows a correlation with its shorter diameter, so that either of

these measurements gives a fair idea of the size of the whole organ. However, in the adult males the shape of the testis changes somewhat with age, becoming relatively longer and more slender. To avoid this source of error, cultures were examined every second day, and males that had hatched in the meanwhile were dissected and measured immediately. Only males that emerged during the first 6 to 8 days of hatching in a given culture bottle were used.

The statistical data obtained are far too voluminous to be published in detail under the present conditions; they are, of course, preserved, and open to all interested. The statistical constants were calculated only for classes in which ten or more individuals were measured.

PRELIMINARY EXPERIMENTS

Theoretically, sterility factors might be located in any of the constituents of the gametes. A few simple experiments of exploratory nature were undertaken to narrow the range of possibilities by excluding some of them as inadequate to explain the situation.

If F_1 hybrid females from the cross A ♀ × B ♂ are used for making backcrosses, the resulting progeny has race A cytoplasm. By crossing such females to race B males, some of the males appearing in the next generation should have all race B chromosomes in race A cytoplasm. If the sterility depends upon an interaction of the chromosomes of one race with the cytoplasm of the other, such males should be sterile. Actually in a number of tests they were found to be fertile. Similarly, males having race A chromosomes in race B cytoplasm proved fertile. This shows that at least the most important sterility factors are not located in the cytoplasm.

To exclude the influence of the Y chromosomes is more difficult. The males devoid of the Y chromosome (XO males) in pure races are sterile, but the structure of their testes shows no similarity whatever with that of the sterile hybrid males (DOBZANSKY 1933). This is in agreement with the results of SHEN (1932) who found that the sterility of XO males in *D. melanogaster* is caused by disturbances in the vesiculae seminales rather than in the testes. Some hybrid males in the backcrosses of hybrid females to race A males are likewise XO. These are, of course, always sterile, but the size, as well as the internal structure of their testes, is variable, just as in their XY sibs. This variability can be due only to the chromosomes other than the Y carried by a given male (DOBZHANSKY 1933). This makes the assumption of factors responsible for hybrid sterility in the Y chromosome unnecessary.

LANCEFIELD (1929) and KOLLER (1932) found that males carrying an X chromosome of one race and a majority of the autosomes of the other race have small testes and are sterile. Furthermore, by studying crossovers

in the X chromosome they found that both ends (or, rather, both limbs) of the X chromosome are concerned with the sterility, while the middle part is apparently not involved. Thus the location of some of the sterility factors was established.

STURTEVANT and the writer attempted to "transfer" mutant genes known in one race into the other race. For this purpose females carrying a given gene, for instance of race A, are repeatedly backcrossed to males of race B, until individuals are obtained possessing all chromosomes of race B except for a more or less short section containing the genes in question. These experiments showed that some of the mutant genes can be thus transferred from race to race, and males carrying these "foreign" genes become fertile after from one to several generations of backcrosses. To this class belong the genes Pointed and short (in the X chromosome of race A), Smoky (second chromosome of race B), Curly (fourth chromosome of B). It follows that sections of chromosomes including these genes contain either no factors concerned with sterility, or else these factors are not by themselves sufficient to produce sterility (although they may, perhaps, do so in combinations with other genes). On the other hand, the attempts to transfer the genes Bare (A) and cinnabar (B) (second chromosome) from one race to another have so far given negative results despite the numerous backcrosses. Similarly, Pointed proved to be closely linked with a sterility-producing section of the X chromosome, but it can be separated from this section by crossing over. Thus, sterility-producing genes were found in both the X chromosome and in an autosome (the second chromosome). The data presented below corroborate this conclusion.

Backcross to race A

Race A females carrying the sex-linked recessives beaded (*bd*), yellow (*y*), and short (*s*), the second chromosome dominant Bare (*Ba*), and the third chromosome recessive purple (*pr*) were crossed to race B males carrying the third chromosome recessive orange (*or*) and the fourth chromosome dominant Curly (*Cy*). F_1 hybrid females heterozygous for these genes were selected and backcrossed to race A males homozygous for *or* and *pr*. The males coming from this backcross were classified for all of the above genes.

According to the setting of the experiment, every one of the chromosomes of the F_1 females (with the exception of the small fifth chromosome in which no genes are available) is marked by one or more genes, which should make the different classes of the backcross males distinguishable from each other phenotypically. Unfortunately, the control of all the chromosomes which is thus attained is far from complete, due to crossing over which takes place in the hybrid females. The X chromosome is

marked by three genes, the third by two, and the second and fourth by only one each. The scarcity of marking genes is mitigated by the fact that the X, second, and third chromosomes of race A differ from the corresponding B chromosomes by inverted sections (TAN 1935 a, b) which suppress a part of the normal crossing over. Nevertheless, some crossovers undoubtedly escape detection.

TABLE 1

Length of the testis (in μ) in the offspring of the cross: (bd y s Ba pr Race A ♀ × or Cy Race B ♂) F1 ♀ × or pr Race A ♂.

PHENOTYPE	M±m	n	PHENOTYPE	M±m	n
1 *bd y s Ba pr*	668.2	9	33 *bd y Ba pr*	348.0	1
2 *bd y s Ba pr Cy*	563.8	8	34 *bd y Ba py Cy*	342.8	3
3 *bd y s Ba or*	585.9±14.9	23	35 *bd y Ba or*	478.5	6
4 *bd y s pr*	632.8±11.0	30	36 *bd y pr*	504.6	4
5 *bd y s Ba or Cy*	509.6±17.6	19	37 *bd y Ba or Cy*	407.2	9
6 *bd y s pr Cy*	602.7± 3.4	28	38 *bd y pr Cy*	435.0	3
7 *bd y s or*	551.2± 6.1	119	39 *bd y or*	462.8	8
8 *bd y s or Cy*	526.5± 8.9	66	40 *bd y or Cy*	418.1±24.4	17
9 *or Cy*	123.9± 2.8	336	41 *s or Cy*	290.2±18.1	22
10 *or*	113.1± 3.2	353	42 *s or*	310.9±18.6	27
11 *pr Cy*	94.0± 3.6	152	43 *s pr Cy*	231.4	4
12 *Ba or Cy*	66.6± 4.7	69	44 *s Ba or Cy*	191.4	1
13 *pr*	68.4± 3.7	159	45 *s pr*	187.9	5
14 *Ba or*	60.2± 4.6	71	46 *s Ba or*	34.8	1
15 *Ba pr Cy*	51.7± 5.9	38	47 *s Ba pr Cy*		
16 *Ba pr*	23.8± 7.3	23	48 *s Ba pr*		
17 *bd y s Ba*	640.3	5	49 *bd y Ba*	461.1	2
18 *bd y s Ba or pr*	664.7	4	50 *bd y Ba or pr*		
19 *bd y s Ba Cy*	553.3	5	51 *bd y Ba Cy*		
20 *bd y s Ba or pr Cy*			52 *bd y Ba or pr Cy*		
21 *bd y s*	597.2±10.4	22	53 *bd y*	574.2	1
22 *bd y s or pr*	617.7±18.4	10	54 *bd y or pr*	614.2	3
23 *bd y s Cy*	559.8±15.6	24	55 *bd y Cy*	461.1	2
24 *bd y s or pr Cy*	494.2	6	56 *bd y or pr Cy*	626.4	1
25 *Cy*	122.8± 4.8	82	57 *s Cy*	274.9	4
26 *or pr Cy*	94.1±10.3	35	58 *s or pr Cy*	487.2	1
27 wild-type	110.7± 5.0	86	59 *s*	261.0	6
28 *or pr*	69.8 ± 6.7	33	60 *s or pr*	104.4	1
29 *Ba Cy*	43.5± 7.3	19	61 *s Ba Cy*		
30 *Ba or pr Cy*	34.8	5	62 *s Ba or pr Cy*		
31 *Ba*	17.1± 4.6	21	63 *s Ba*	87.0	2
32 *Ba or pr*	47.0	3	64 *bd or*	574.2	1

In spite of the above difficulty, it is believed that the control of the chromosomes, incomplete as it is, is on the whole adequate for our purpose, namely testing for the presence or absence of the sterility genes in each of the chromosomes covered by the investigation. The basis of our judgement is the mean testis size in the males of a given class showing a given combination of the marking genes. Although some of the chromosomes are

marked by a single gene only, all the males carrying this gene will carry at least a part of the chromosome of the race indicated by this gene, and the majority will carry probably the entire chromosome. In any event,

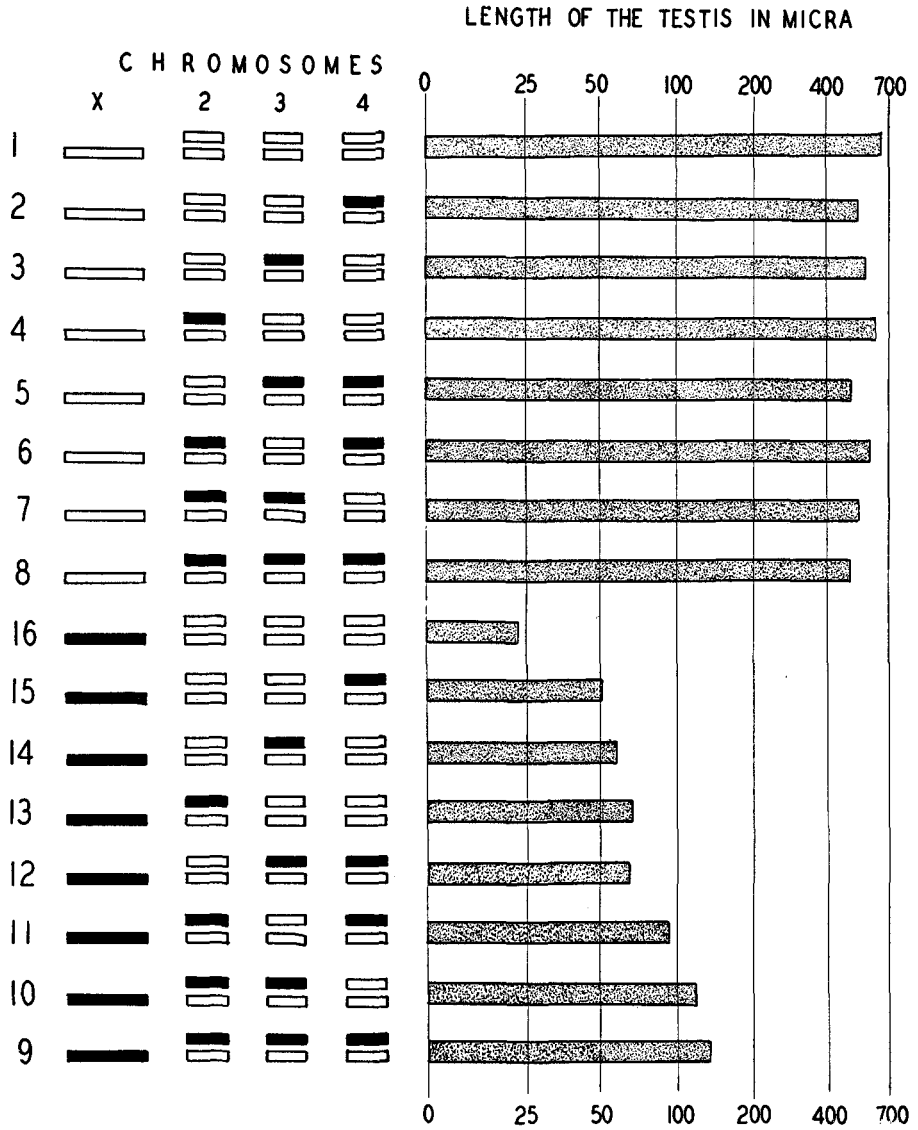

FIGURE 1. The chromosome constitution and the length of the testis in males appearing in the backcross of the F_1 hybrid females to race A males. Race A chromosomes—white; race B chromosomes—black. Only the non-crossover classes are represented.

males of every class have a greater chance to have the chromosomal constitution indicated by the marking genes they carry than any other constitution. In other words, since the conclusions are to be based on averages

and not on individual variants, the disturbing influence of the undetected crossovers will manifest itself in a great variability and not in a distortion of the relationships of different classes. These remarks apply to all the experiments described in this article.

Every individual resulting from a backcross to race A male necessarily has one whole complement of autosomes from race A. All males have also a Y chromosome from race A. However, some of them have a race A X chromosome, and others a race B X chromosome. They inherit from their mothers one or more autosomes of race A, or of race B. Not counting crossovers, and disregarding the fifth chromosome, the backcross males should fall into sixteen classes (classes 1–16 in table 1, figure 1).

Males receiving race A X chromosomes (classes 1–8, 17–24) have distinctly larger testes than those receiving race B X's (classes 9–16, 25–32). It follows that the X chromosome carries genes concerned with the sterility. Next, one may notice that different classes of males carrying the same X chromosome vary greatly as to testis size. Classes possessing the B race X chromosome may be taken up first. Among them the largest testes are observed in individuals having one full set of race B autosomes (class 9, also 25 and 26). Making such males homozygous for the race A fourth chromosome decreases testis size (compare classes 9 and 10, 25 and 27, 26 and 28). The differences are not in all cases statistically significant, but they are always in the same direction. Homozygosis for race A third chromosome also decreases testis size, the effect of the third chromosome being stronger than that of the fourth (compare classes 9 and 11). The race A second chromosome produces a still stronger effect in the same direction (compare 9 and 12, 25 and 29, 26 and 30). The third and the fourth chromosomes of race A being homozygous simultaneously produce as much effect as the second alone (12 and 13). Homozygosis for second and fourth, and second and third depresses testis size still further (14 and 15). Finally, males possessing only a race A autosomes have the smallest testes (class 16). The conclusion is warranted that in males carrying race B X chromosome the testes are larger the more B autosomes they carry (although, as stated above, all these males carry one full set of A autosomes).

The mean values for testis size in males having race A X chromosome are based on a smaller number of flies than those discussed above. Nevertheless, it is quite clear that in these males the relations are reversed, namely the testes are the larger the more race A autosomes they carry. The largest testes are observed in males carrying all or most of race A autosomes (classes 1, 17, 18), and the smallest in males having a full set of B autosomes (8, 23, 24). Hence, a general rule may be formulated thus: in the backcross males the testes are larger the more the autosomes agree

in their racial origin with the X chromosome, and vice versa. The second, third, and fourth chromosomes are all concerned, but to a different extent: the second chromosome exerts the strongest effect, the third is next, and the fourth last.

Since two of the chromosomes in this experiment contain more than one marking gene each, some crossovers are detected. This makes it possible to determine (a) whether these chromosomes contain more than one locus concerned with testis size, and (b) if this be the case, which part of a given chromosome exerts a stronger effect.

Three genes, *bd*, *y* and *s*, are present in the X chromosome. Crossing over between *bd* and *y* has been observed in our hybrids only once (class 64), although about 30 percent of crossing over takes place between these genes in pure race A (this is due to the inversions in the left limb of the X suppressing crossing over, TAN 1935). On the other hand, crossing over between *y* and *s* is fairly frequent (though less frequent than in pure race A, where these loci are practically independent). Classes carrying the whole X chromosome of race A (*bd y s*, classes 1–8, 17–24) may be compared with those having the right limb, or a part thereof, of race B (*bd y*, classes 33–40, 49–56). If this comparison is made so that classes differing only in the substitution of the right limb of the X are considered (classes 1 and 33, 2 and 34, 17 and 49, 24 and 55, etc.), the conclusion is that such a substitution decreases testis size. This is observed in twelve out of thirteen such comparisons. On the other hand, classes carrying the whole X chromosome of race B (non-*bd*, non-*y*, non-*s*) may be compared with those having the right limb of the X of race A (classes showing *s*, compare 9 and 41, 10 and 42, 25 and 57 etc.). The comparison shows that in this case the substitution of the right limb invariably results in a marked increase of the testis size. The conclusion follows that the right limb, as well as the left limb, of the X chromosome carry genes concerned with sterility.

In order to compare the relative efficacy of the two limbs of the X chromosome, classes carrying the left limb of race A and the right limb of race B must be compared with those having the left limb of B and the right limb of race A. The classes to be compared should, of course, have identical autosomes (compare 35 and 46, 36 and 45, 37 and 44, 38 and 43, 39 and 42, 40 and 41). It can be seen that classes having the left limb of race A have larger testes, and hence the left limb is more important than the right.

An analysis of the third chromosome, which in this experiment is marked by two genes, *or* and *pr*, can be carried through along lines similar to those for the X chromosome (compare classes 1–8 with 17–24, 9–16 with 25–32, and then 17 with 18, 21 with 22, 25 with 26 etc.). Since the

effect of the third chromosome as a whole on testis size is much weaker than that of the X chromosome, the analysis of the effect of the former is more difficult. Nevertheless, the majority of the figures indicate that (a) both the part of the third chromosome carrying *or* and that carrying *pr* are concerned with testis size, and (b) that the part containing *pr* exerts a greater effect than that containing *or*.

TABLE 2

Length of the testis (in μ) in the offspring of the cross:
(bd y s Ba Race A ♀ × or Cy Race B ♂) F_1 ♀ × or Race B ♂

CLASS NO.	PHENOTYPE	M ± m	σ	LIMITS	n
1	*or Cy*	475.4 ± 4.3	60.7	244–661	200
2	*or*	468.8 ± 4.3	60.0	244–661	200
3	*Cy*	565.3 ± 3.9	55.2	313–748	200
4	*Ba or Cy*	375.0 ± 4.5	57.1	174–557	158
5	wild type	561.5 ± 4.1	59.0	296–730	204
6	*Ba or*	336.5 ± 4.7	60.9	157–609	168
7	*Ba Cy*	393.8 ± 5.7	67.0	191–644	138
8	*Ba*	372.2 ± 4.7	56.7	157–574	144
9	*bd y s Ba*	428.6 ± 9.2	61.6	278–592	45
10	*bd y s Ba Cy*	325.6 ± 11.7	61.8	139–470	28
11	*bd y s Ba or*	278.4 ± 8.9	53.2	139–435	36
12	*bd y s*	156.8 ± 5.9	55.0	70–313	86
13	*bd y s Ba or Cy*	211.4 ± 5.9	39.8	70–365	46
14	*bd y s Cy*	123.0 ± 3.7	39.0	35–244	111
15	*bd y s or*	109.8 ± 3.6	37.8	35–261	108
16	*bd y s or Cy*	81.4 ± 3.6	35.7	0–157	98
17	*s or Cy*	284.5 ± 7.9	55.9	139–505	52
18	*s or*	300.0 ± 6.9	58.8	122–487	73
19	*s Cy*	458.0 ± 10.4	60.0	296–644	33
20	*s Ba or Cy*	257.9 ± 15.5	67.7	104–418	19
21	*s*	468.8 ± 8.5	59.9	313–661	50
22	*s Ba or*	330.9 ± 13.8	66.5	122–487	23
23	*s Ba Cy*	324.0 ± 14.0	56.0	209–470	16
24	*s Ba*	426.3 ± 14.9	64.9	278–574	19
25	*bd y Ba*	488.4 ± 14.3	53.2	383–644	14
26	*bd y Ba Cy*	453.1 ± 22.1	79.7	244–626	13
27	*bd y Ba or*	313.2		313	2
28	*bd y*	258.6 ± 13.5	63.5	122–435	22
29	*bd y Ba or Cy*	208.8		139–365	8
30	*bd y Cy*	198.5 ± 11.3	37.4	157–313	11
31	*bd y or*	115.4 ± 12.6	48.7	52–313	15
32	*bd y or Cy*	119.2		70–278	8

Backcrosses to race B

Race A females carrying the genes beaded, yellow, short, Bare and purple (*bd y s Ba pr*) were crossed to race B males carrying orange and Curly (*or Cy*). In the F_1 generation females heterozygous for all these genes were selected, and backcrossed to race B males homozygous for orange. The experiment is, then, analogous to that described above, with the exception that since the male to which the hybrid females are backcrossed

belongs to race B, all the offspring appearing in the next generation will necessarily carry one complete set of B race (instead of A race as in the preceding experiment) autosomes, and some of them may carry B race chromosomes exclusively. Moreover, the gene purple does not manifest

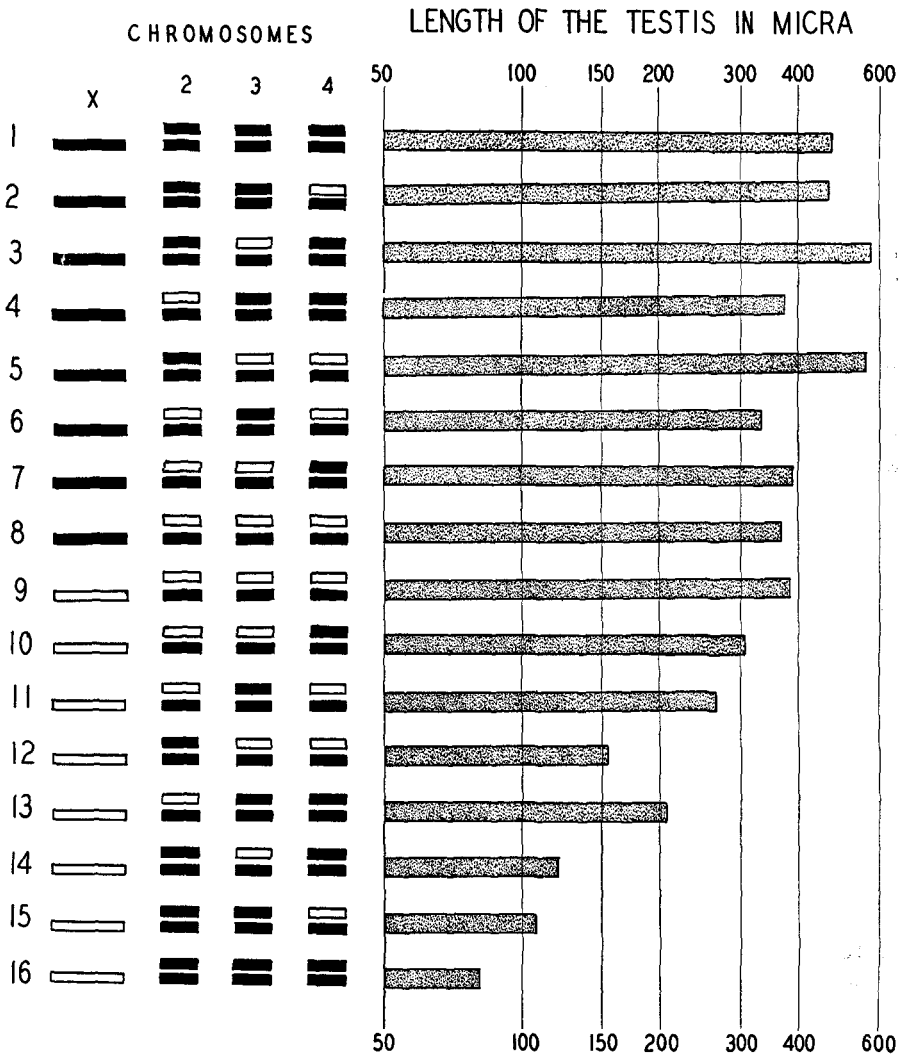

FIGURE 2. The chromosome constitution and the length of the testis in males appearing in the backcross of the F_1 hybrid females to race B males. Race A chromosomes—white; race B chromosomes—black. Only the non-crossover classes are represented. (The bar for class 9 should extend to 428.6; see table 2.)

itself in the offspring of the backcross, since the father of the backcross is homozygous for the wild type allelomorph of this gene.

The measurements of testis size in the males obtained from this backcross are summarized in table 2 and figure 2. Among the classes in which no crossovers are detected (classes 1–16), the first eight (1–8) carry the

X chromosome of race B, and, with one exception, have distinctly larger testes than the classes (9–16) carrying the X chromosome of race A. Thus, with a majority of the autosomes coming from race B, a race B X chromosome is required if large testes are to be formed—a result completely analogous, but reverse in sign, to that arrived at above by studying the backcross to race A males.

Among the males carrying the same X chromosome, the size of the testes depends upon the autosomes. Males receiving an X chromosome of race A (classes 9–16) have the largest testes if they carry one complete set of race A and one set of race B autosomes (class 9). If such males are made homozygous for race B fourth chromosome, the testis size is decreased (compare 9 and 10, 11 and 13, 12 and 14, 15 and 16). A similar, but stronger, effect is produced by homozygosis for the third chromosome of race B (compare 9 and 11, 10 and 13, 12 and 15, 14 and 16), and, finally, the second chromosome exerts the strongest effect (classes 9 and 12, 10 and 14, 11 and 15, 13 and 16). The conclusion is warranted that males having the race A X chromosome have the larger testes the more race A autosomes they carry.

By analogy with the conclusion just stated, as well as with the conclusion arrived at from the study of the backcross to race A, one might expect that in the present experiment males carrying race B X chromosome should have the larger testes the more race B autosomes they carry. Indeed, this is partly, but only partly, true. Among such males, those that are homozygous for race B fourth chromosome have larger testes than those carrying one race A fourth chromosome (compare classes 1 and 2, 3 and 5, 4 and 6, 7 and 8), and those homozygous for race B second chromosome have larger testes than those carrying one race A second chromosome (compare 1 and 4, 2 and 6, 3 and 7, 5 and 8). Turning to the third chromosome, one is at once struck by the fact that it shows a relationship that is the reverse of the expected one: males homozygous for the race B third chromosome have smaller testes than those possessing one third chromosome of race A (compare 1 and 3, 2 and 5, 4 and 7, 6 and 8). This inconsistency is to be discussed below.

Crossing over between *y* and *s* in the X chromosome produces males carrying an X the right limb of which (or a part thereof) comes from one race, and the left limb from the other race. One may observe that males having the right limb of the X chromosome from race A, and the left from race B (showing the effects of the gene *s*, but not of *bd* or *y*, in their phenotype), have mostly smaller testes than those carrying an intact X chromosome of race B (compare classes 17–24 with 1–8). Conversely, males carrying the right limb of the X from race B and the left from race A (showing *bd* and *y*, but not *s*), have larger testes than males with

complete race A X chromosome (compare 25–32 with 9–16). Finally, with the autosomes held constant, males having the right limb of the X from race A and the left limb from race B have mostly larger testes than males carrying the right limb of the X from B and the left from A (compare classes 17 and 32, 18 and 31, 19 and 30, etc.). It follows from this that (a) both limbs of the X chromosome carry sterility genes, and that (b) the sterility genes in the left limb are either more numerous or more effective than those in the right limb. Both conclusions are indentical with those reached from the study of the backcross to race A.

Returning again to the unexpected behavior of the third chromosome in males carrying the X chromosome of race B (see above), one might notice that in the experiment under consideration the cross was so arranged that all the hybrids originated from race A females (*bd y s Ba pr*), and, consequently, have race A cytoplasm. This fact may arouse the suspicion that homozygosis for the race B third chromosome is incongruent with race A cytoplasm, in such a way that in males carrying race A cytoplasm and all or a majority of race B chromosomes, the presence of one race A third chromosome may increase, instead of decrease, the testis size. If this were true, we would have the first indication that the cytoplasms of races A and B are specifically distinct and may be concerned with the production of the sterility of the hybrids. The possibility just stated can be subjected to a rigorous experimental test: a cross must be arranged which should produce offspring completely comparable with those from the cross just discussed in the chromosomal constitution, but which should have throughout the cytoplasm of race B, instead of that of race A. This requirement is fulfilled in the following experiment.

Race B females carrying *or* and C*y* were crossed to *bd y s Ba pr* males of race A. F_1 females heterozygous for all these genes were backcrossed to race B males homozygous for *or*. All the strains used in this cross were the same as those used in the preceding experiments. The results are summarized in table 3. They should be compared to those presented in table 2. In these tables, the classes showing identical phenotypes have similar chromosomal constitutions, but differ in that the data of table 2 pertain to flies having race A cytoplasm, and those of table 3 to flies having race B cytoplasm. It is easily noticeable that the corresponding classes of males shown in table 2 have consistently larger testes than those shown in table 3. However, this fact is probably of no particular significance, since the experiments on which tables 2 and 3 are based were done at different times, and, due to the extreme sensitivity of the testis size to environmental conditions, no two separate experiments are likely to agree as far as the absolute size of the testes of hybrid males is concerned. Far more consequential is the relative testis size in males of different classes in each

TABLE 3

Length of the testis (in μ) in the offspring of the cross: (or Cy Race B ♀ × bd y s Ba Race A ♂) F_1 ♀ × or Race B

CLASS NO.	PHENOTYPE	M ± m	σ	LIMITS	n
1	*or Cy*	401.6 ± 7.2	72.9	226–609	103
2	*or*	404.9 ± 6.5	68.0	261–557	109
3	*Cy*	541.7 ± 7.4	80.6	313–731	118
4	*Ba or Cy*	298.6 ± 6.4	77.6	122–470	146
5	wild type	518.0 ± 6.6	71.9	348–679	120
6	*Ba or*	296.5 ± 6.3	72.4	139–487	133
7	*Ba Cy*	342.6 ± 7.7	82.7	157–522	114
8	*Ba*	359.1 ± 8.3	89.6	191–557	116
9	*bd y s Ba*	283.4 ± 10.3	63.2	174–452	38
10	*bd y s Ba Cy*	273.9 ± 9.6	64.6	174–400	45
11	*bd y s Ba or*	251.8 ± 7.8	62.1	139–383	63
12	*bd y s*	108.4 ± 3.2	41.9	35–261	171
13	*bd y s Ba or Cy*	194.5 ± 7.3	61.9	70–331	71
14	*by y s Cy*	79.9 ± 2.5	31.5	35–157	164
15	*bd y s or*	57.8 ± 2.6	31.5	17–139	149
16	*bd y s or Cy*	41.6 ± 2.5	32.7	0–139	177
17	*s or Cy*	213.7 ± 9.9	66.5	70–348	45
18	*s or*	206.4 ± 8.6	57.8	104–348	45
19	*s Cy*	330.1 ± 10.7	66.1	191–505	38
20	*s Ba or Cy*	179.7 ± 14.6	70.1	104–400	23
21	*s*	290.1 ± 11.8	67.7	174–417	35
22	*s Ba or*	195.4 ± 8.8	47.9	104–331	30
23	*s Ba Cy*	313.2		261–418	7
24	*s Ba*	252.3 ± 14.7	62.1	174–417	18
25	*bd y Ba*	464.1 ± 36.7	127.4	278–679	12
26	*bd y Ba Cy*	461.1 ± 22.5	74.1	278–557	11
27	*bd y Ba or*	438.0 ± 18.4	71.5	313–557	15
28	*bd y*	241.3 ± 10.4	67.9	122–418	43
29	*bd y Ba or Cy*	402.6 ± 13.3	44.0	331–47	11
30	*bd y Cy*	194.9 ± 8.9	56.4	87–365	40
31	*bd y or*	140.4 ± 7.7	49.9	52–261	42
32	*bd y or Cy*	122.0 ± 6.5	40.7	70–244	39
33	*y s*	643.8		626–661	2
34	*bd*	87.0		87	1
35	*bd Cy*	417.6		417.6	1
36	*y s or Cy*	556.8		556.8	1

experiment. Approaching the data from the standpoint of this criterion, one is forced to the conclusion that the data of tables 2 and 3 are identical in all essentials. For our purposes, the most important fact is that in males having race B cytoplasm and race B X chromosome, homozygosis for the race B third chromosome produces a decrease, instead of an increase, of the testis size. Hence, the anomalous behavior of the third chromosome in these crosses cannot be due to an interaction between this chromosome and the cytoplasm. No indication of the existence of an inherent difference between the cytoplasms of the two races is apparent.

Obviously, an explanation of the anomalous effect of the third chromosome in the crosses under consideration is to be looked for elsewhere. Two such explanations may be suggested. First, one may suppose that it is a specific property of the third chromosome of race A to increase the testis size in males having a majority of the chromosomes of race B. In this case, a male of hybrid ancestry having one race A third chromosome and the rest of the chromosomes of race B would always have larger testes than pure race B males. Second, the above behavior of the third chromosome may be due to a maternal effect, being manifested only in flies coming from the eggs of a hybrid mother. This amounts to assuming that the presence of the hybrid karyotype in the egg cell, before it has undergone the processes of maturation and fertilization, leaves an impression on this cell that lasts for at least one generation.

The two alternative explanations just suggested are obviously *ad hoc* hypotheses, and should be considered objectionable on this ground, were it not for the fact that they may be tested experimentally. Males of class 3 (table 3) have all the chromosomes of race B except a single race A third chromosome. Their race B third chromosome carries the marking gene *or*. Having large testes, these males are fertile, and can be crossed to pure B females homozygous for *or*. In the offspring of this cross two classes of males must appear. One of them, phenotypically orange, will have only race B chromosomes, and will be genotypically identical with males of classes 1 and 2 in table 3. The other class will be wild type in phenotype, and will be genotypically identical with class 3 in table 3, that is, will carry one race A third chromosome. Now, if the third chromosome of race A has *per se* the property of increasing testis size in males that are otherwise race B in constitution, the wild type males in this experiment should have larger testes than the orange males. Thus, the relationships observed in the first backcross generation (tables 2 and 3) should be repeated in the next generation. If, on the other hand, the phenomenon under consideration is due to a maternal effect, the testes of the wild type and the orange males should be either equal in size, or else the orange males should have larger testes than the wild type ones.

The experiment has been arranged as just outlined, and the testes in the resulting males were measured. Their size (in μ) was:

orange	607.6 ± 8.6
wild type	578.6 ± 7.4

The difference between these figures is not statistically significant, but the orange males have testes either equal to or larger than the wild type ones. The maternal effect hypothesis is correct. Maternal effects have been observed in *Drosophila pseudoobscura* crosses more than once (DOBZHANSKY 1935, DOBZHANSKY and STURTEVANT 1935).

STERILITY GENES IN THE SECOND CHROMOSOME

The experiments reported above show that genes responsible for the sterility of the interracial hybrids are located in the X, second, third, and fourth chromosomes—all chromosomes except the fifth, which has not been followed in the crosses. Furthermore, it has been shown that in the X and also in the third chromosome more than one sterility gene is present, located in different parts of the respective chromosomes. The question whether the second chromosome has one or more sterility genes could not be answered, since this chromosome carried a single gene marker, namely Bare. In the experiment now to be discussed this drawback is removed by introducing two marking genes in the second chromosome.

It may also be noticed that in the former experiments a majority of the marking genes were introduced through the race A parent, causing the classes of the offspring having mainly A race chromosomes to appear in relatively low frequencies. In the following experiment mainly race B markers are used.

Race B females carrying the sex-linked recessives scutelar (*sc*) and dela (se^a), and the second chromosome recessive cinnabar (*cn*) and dominant Smoky (*Sm*), were crossed to race A males carrying the fourth chromosome dominant Curly (*Cy*). The F_1 females heterozygous for these genes were backcrossed to race B cinnabar males. The results are summarized in table 4. The gene Curly involved in this cross was originally obtained as a mutant in race B, and "transferred" into race A by means of repeated backcrosses of Curly flies to race A males (see above). Thus, the race A fourth chromosome marked by *Cy* in this experiment is really a composite chromosome, containing, presumably, most of the material from race A, and a more or less small section including the locus of the gene *Cy* from race B.

To start with, one may notice that the data of table 4 corroborate the general conclusions regarding the action of the sterility genes previously discussed. Since males recorded in table 4 have at least one full set of race B autosomes, the classes carrying an intact race B X chromosome (1–4, 17–20) have larger testes than those carrying an intact X of race A (5–8, 21–24). Among males carrying the same X chromosome, largest testes are present in those that have most autosomes of the same race as the X chromosome (class 1—all chromosomes of race B, class 5—race A X chromosome and one set of A autosomes), and smallest testes in males having an X of one race and the autosomes of the other (class 4 with X of race B and one set of race A autosomes, class 8 with X of race A and race B autosomes). The fourth chromosome marked by *Cy* behaves as a race A chromosome in spite of the fact that it carries a section coming from race B. Crossing over in the X chromosome leads to the results expected on

the basis of the previously reported experiments: males having the left part of the X from race B (carrying *sc*) have larger testes than males having the right part of the X from race B but otherwise similar (males showing *se* but not *sc*).

TABLE 4

Length of the testis (in μ) in the offspring of the cross:
(sc se cn Sm Race B ♀ × Cy Race A ♂)F₁ ♀ × cn Race B ♂.

CLASS NO.	PHENOTYPE	M ± m	σ	LIMITS	n
1	*sc se cn Sm*	563.6 ± 6.7	56.6	418–679	103
2	*sc se cn Sm Cy*	523.2 ± 10.3	76.9	400–714	72
3	*sc se*	449.6 ± 10.6	84.4	278–626	61
4	*sc se Cy*	420.7 ± 9.3	70.1	261–592	56
5	*Cy*	319.5 ± 7.2	76.2	122–522	114
6	wild type	282.6 ± 6.1	67.0	122–435	122
7	*cn Sm Cy*	97.1 ± 4.0	36.9	35–174	85
8	*cn Sm*	90.7 ± 4.4	40.9	17–209	87
9	*sc cn Sm*	455.9 ± 10.0	58.3	365–592	34
10	*sc cn Sm Cy*	416.6 ± 12.5	70.5	226–592	32
11	*sc*	426.0 ± 10.0	74.1	261–592	55
12	*sc Cy*	435.4 ± 8.8	68.2	296–592	60
13	*se Cy*	433.3 ± 10.4	66.1	278–557	40
14	*se*	420.0 ± 11.7	78.0	278–592	45
15	*se cn Sm Cy*	120.6 ± 9.7	51.7	52–244	28
16	*se cn Sm*	86.0 ± 5.7	32.2	0–174	32
17	*sc se cn*	578.6 ± 9.2	63.3	452–661	48
18	*sc se Sm*	371.0 ± 9.7	60.9	209–487	39
19	*sc se cn Cy*	554.0 ± 12.6	78.7	388–696	39
20	*sc se Sm Cy*	364.2 ± 10.5	55.7	226–452	28
21	*Sm*	241.2 ± 9.9	74.1	87–452	56
22	*cn*	99.0 ± 4.5	33.1	52–243	54
23	*Sm Cy*	248.8 ± 9.0	69.4	104–418	60
24	*cn Cy*	121.3 ± 7.5	46.1	0–226	28
25	*sc cn*	492.9 ± 11.2	66.1	348–626	35
26	*sc Sm*	407.5 ± 11.8	60.4	313–557	26
27	*sc cn Cy*	500.6 ± 18.6	86.8	261–609	22
28	*sc Sm Cy*	396.5 ± 11.9	63.2	296–505	28
29	*se cn*	102.3 ± 7.8	39.8	52–191	26
30	*se Sm*	401.4 ± 12.8	61.6	226–487	23
31	*se cn Cy*	116.8 ± 8.8	43.0	70–261	28
32	*se Sm Cy*	463.9 ± 18.3	79.3	296–626	19

The detected crossing over in the second chromosome gives rise to males showing *Sm* but not *cn*, and *cn* but not *Sm* (classes 17–32). Males carrying the X and the second chromosomes of race A (classes 5 and 6) have larger testes than the corresponding classes of males in which a part of the second chromosome of race B is present (classes 21–24), but the latter have larger testes than males carrying the X of race A and the whole second chromosome of race B (classes 7–8). Males possessing the X and the second chromosomes of race B (classes 1 and 2) have testes equal to or larger than

similar males carrying a part of the second chromosome of race A (classes 17–20). In either case, the substitution of the right part of the second chromosome (carrying *Sm*) produces less effect than the substitution of the left part of the second chromosome (carrying *cn*).

The conclusion is warranted that more than one locus concerned with sterility is present in the second chromosome, and that the part of the chromosome marked by *cn* carries either more numerous or stronger sterility factors than the part marked by *Sm*. This result was not unexpected, in view of the fact that, according to the data of STURTEVANT, the gene *Sm* is easily transferred from race B to race A, whereas we have not been able to transfer *Ba* (lying very close to *cn*) from race A to race B. A less extensive series of attempts to transfer *cn* from race B to A was also unsuccessful.

Since the real position of the genes *cn*, *Ba*, and *Sm* in the second chromosome (in terms of the cytological map) is as yet unknown, it is not possible to decide which of the two parts of the chromosome is longer, and hence it remains obscure whether or not the effectiveness of a given chromosome section is proportional to its cytological length.

FERTILITY TESTS

The main body of our conclusions regarding the localization of the sterility genes is based on testes measurements, not on direct fertility tests. The justification of the procedure is given above, and need not be repeated here. Some fertility tests were performed as an additional check.

In the offspring of the backcross to race B the results of which are represented in table 2 males of the classes 1–8 were selected and crossed to pure race B females. Fifty males of each class were segregated into batches of five, and each batch was placed with 3–4 females in a separate culture bottle. No tests of individual males were made, since the males appearing in the backcrosses are, in contradistinction to males of pure races or the F_1 hybrids, rather weak, sometimes somatically abnormal, and in general inferior in vigor. The causation of this decrease in vigor in backcross males constitutes a separate problem (DOBZHANSKY and STURTEVANT 1935).

Males of the classes 5, 6, 7 and 8 produced no offspring. Hence, males carrying a second chromosome of race B and an X of race A are always sterile (which was to be expected since they have testes distinctly smaller than normal). All culture bottles containing males of the classes 1, 2, 3, and 4 produced offspring, although some of them did so only after repeated transfers on fresh food, and even then the offspring were few in number. The conclusion follows that males carrying all race B chromosomes in race A cytoplasm may be fertile, and the presence of the race A third or fourth chromosomes, or both together, with race B X and second chromosomes, does not necessarily prevent fertility.

THE SECOND BACKCROSS GENERATION

Females appearing in the first backcross generation fall into as many distinct genotypic classes as do their brothers. All of them possess a full set of the chromosomes of the race to which their father belonged, but the second set of the chromosomes inherited from the mother may be of either race, or may be partly derived from one race and partly from the other.

Some females that were sisters of the males whose testis measurements are presented in table 2 (coming from a backcross of the F_1 hybrid females to race B males) were individually crossed to race B males homozygous for orange. Since these females have their race A chromosomes marked by mutant genes, their own genetic constitution, as well as the genetic constitution of their male offspring, can be judged by the phenotype. No testis measurements were made on the males of the second backcross generation, but the testis size was evaluated by dissection and a simple inspection.

The results obtained are summarized below.

1. Females carrying all chromosomes of race B (*or Cy* in phenotype). Four such females tested; all sons have testes of normal size.

2. Females carrying an X of race A, rest of the chromosomes race B (*or Cy* in phenotype). One female tested; wild type sons have large testes, *bd y s* sons small to intermediate.

3. Females carrying a race A fourth chromosome, rest of the chromosomes race B (*or* in phenotype). Two tested; all sons have large testes.

4. Females carrying a race A third chromosome, rest of the chromosomes race B (*Cy* in phenotype). Five tested; four produced all sons with large testes, one gave sons with testis size varying from large to intermediate.

5. Females as above, but also one race A X chromosome (*Cy* in phenotype). Two tested; *bd y s* sons have small testes, wild type ones large testes.

6. Females carrying one third and one fourth chromosome of race A, the rest of the chromosomes of race B (wild type in phenotype). Seven tested; two produced sons with large testes, and five gave sons with testes varying from large to intermediate.

7. Females as above, but also one race A X chromosome (wild type in phenotype). One tested; *bd y s* sons had very small testes, non-*y* sons large to intermediate.

8. Females having one second and one fourth chromosomes of race A, the rest of race B (phenotype *Ba or*). One tested; *Ba* sons with very small testes, non-*Ba* sons with testes of intermediate size.

9. Females having one second and one third chromosomes of race A, the rest of race B (phenotype *Ba Cy*). Four tested; wild type and *Cy* sons had

testes of intermediate size, *Ba Cy* sons—small testes, *Ba* sons—very small testes.

10. Females having one set of autosomes of race A, the X's and the other set of autosomes of race B (phenotype *Ba*). One tested; wild type sons had intermediate or small testes, *Ba* sons very small testes.

These data, meager as they are, corroborate the conclusions regarding the distribution of the sterility factors in the chromosomes reached in the main body of the work. The failure to test a large number of the backcross females is due to the fact that they are on the whole much weaker than the F_1 hybrid females, and frequently produce no offspring.

DISCUSSION

The possibility that the sterility of the interracial hybrids in *Drosophila pseudoobscura* is due to an accumulation of structural differences between the chromosomes of the two races may be considered excluded. Some arguments against this possibility were presented in an earlier publication (DOBZHANSKY 1934). More recently TAN (1935a, b) has studied the chromosomes of the salivary gland cells in both races, and has found that they differ in six inverted sections, four of which are located in the X chromosome, one in the second and one in the third. Since in *D. pseudoobscura* the sterility is confined to the male hybrids, only the two autosomal inversions come under consideration as a possible cause of sterility. *D. melanogaster* individuals of either sex heterozygous for five inversions (delta-49 in the X chromosome, CIIL and CIIR of Curly in the second, CIIIL and CIIIR of Deformed in the third chromosome) are fertile. According to the unpublished data of STURTEVANT, the wild strains of *D. pseudoobscura* found in nature are frequently heterozygous for inverted sections in the third, and less frequently also in the second and in the X chromosomes, but no sterility is apparent in these strains. The autosomal interracial inversions discovered by TAN are remarkable neither in their cytological length nor in the extent of the suppression of crossing over they produce in the chromosomes concerned. The fourth chromosomes of the two races are, according to TAN, similar in gene alignment, but a failure of their pairing is frequently observed in the interracial hybrids. In short, the evidence against these inversions directly causing the sterility in *D. pseudoobscura* appears conclusive.

The data presented in this article show that genetic factors causing sterility of the interracial hybrids exist in all the chromosomes tested, that is in all the chromosomes except the fifth. Moreover, in the X, the second, and the third chromosomes respectively more than one sterility factor was detected. On the other hand, the effects of the different chromosomes are not equally strong: the left limb of the X has the strongest effect, the

right limb of the X, the second, the third, and the fourth chromosomes are decreasingly effective in the order indicated. This fact may be interpreted as indicating that "main sterility factors" lie in the X and in the second chromosomes, while the third and the fourth contain minor contributing factors or modifiers. This interpretation is, however, not a necessary one. The cytological lengths of the chromosomes (in salivary gland cells, according to TAN) decrease in the following order: X chromosome, second, third, and fourth chromosomes. Hence, the effectiveness of a chromosome in producing sterility is on the whole proportional to its length. That no strict proportionality of this sort obtains is clearly demonstrated by the greater effectiveness of the shorter left limb of the X as compared with the cytologically longer right limb of the same chromosome, but the available data permit no classification of the sterility factors into main and modifying ones. The greater effectiveness of the part of the second chromosome carrying the loci of Bare and cinnabar as compared with the part of the same chromosome carrying Smoky also gives no evidence on this point since the cytological lengths of these parts are as yet unknown. Likewise, the question of whether or not the parts of the chromosomes carrying interracial inversions are especially likely to contain numerous or powerful sterility genes remains open.

The available data are best interpreted as meaning that the testis size in the backcross hybrids is the larger the more their X chromosome agrees with the autosomes as to its racial origin. In other words, sterility versus fertility seems to be determined by interactions of factors located in the X chromosome with factors located in the autosomes. Here again, this interpretation is not a necessary one, since the cytological length of the X of *D. pseudoobscura* is almost equal to the sum of the lengths of all the autosomes. Hence, the data are not inconsistent with the supposition that testes are smallest in males carrying equal volumes of the chromosomal material from both races, and that the more homogeneous are the chromosomes in their racial origin, the larger are the testes.

Concerning the mechanism of the action of the sterility factors, the available data permit a single conclusion only, namely that their effects on testis size are additive. This is amply demonstrated by the figures in tables 1–4: if two chromosomes, or sections of chromosomes, each produce a decrease (or an increase) in testis size, these two chromosomes, or sections, produce a larger decrease (or increase) if they are present simultaneously. More complicated forms of interactions, for instance factors whose effects are contingent on the presence of other factors, have not been detected. The only exception is the third chromosome of race B, the action of which in the hybrids possessing predominantly race B chromosomes (or at least the race B X chromosome) is complicated by a maternal effect

(see the discussion of this point in the text). It is of interest that in hybrids carrying predominantly race A chromosomes the third chromosome does not show any anomalous effects.

SUMMARY

1. Crosses between race A and race B of *Drosophila pseudoobscura* produce in F_1 fertile daughters and sterile sons. The F_1 females may be backcrossed to males of either parental race. Some of the males in the resulting offspring are fertile and others are sterile. Fertile males always have large testes, while testes in the sterile males vary in size from normal to very small. The testis size is an index of the degree of disturbance in the process of spermatogenesis, the disturbance being greatest in the smallest testes.

2. Backcross males having only race A chromosomes are fertile irrespective of whether they have the cytoplasm derived from race A or from race B. Backcross males having only race B chromosomes are likewise fertile irrespective of the source of their cytoplasm.

3. In the offspring of the backcross of the F_1 hybrid females to race A males, the males carrying the X chromosome from race A have larger testes than those carrying the X chromosome from race B (table 1, figure 1). Among classes carrying the same X chromosome testis size depends upon the autosomes: the more the autosomes agree in their racial origin with the X chromosome the larger are the testes, and vice versa.

4. Backcrosses of the F_1 hybrid females to race B males give results analogous to the above but reverse in sign: the largest testes are present in backcross males having the race B X chromosome and race B autosomes, and smallest testes in males carrying race A X chromosome and B race autosomes (tables 2, 3, and 4, figure 2).

5. All the chromosomes studied carry genes concerned with testis size, and consequently with sterility. The X chromosome produces, however, the strongest effect, the second chromosome follows next, and the third and the fourth chromosomes last. Thus, on the whole, the effectiveness of each of the chromosomes is proportional to its cytological length.

6. Wherever in our experiments a chromosome was marked by more than one mutant gene it was possible to show that sterility genes are present in different parts of this chromosome.

7. The effects of the sterility genes located in the different chromosomes and sections of the chromosomes are additive.

8. The behavior of the third chromosome of race B in the hybrid males of the first backcross generation possessing a majority of race B chromosomes is anomalous. Such males have larger testes if they carry one race A and one race B third chromosome than if they are homozygous for the race B third chromosome. This anomaly is due to a maternal effect: in in-

dividuals coming from the eggs of the F_1 hybrid females homozygosis for the race B third chromosome decreases the testis size.

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