DEFICIENCY AND DUPLICATIONS FOR THE GENE BOBBED IN DROSOPHILA MELANOGASTER

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INTRODUCTION

The present paper consists of two parts. The first deals with a description of a deficiency involving the locus of the sex-linked gene bobbed. The results obtained have a bearing on the problems of the cytological map of the X chromosome and on the existence of an "inert" region in this chromosome. The second part of the paper is devoted to the study of the interactions between bobbed-deficiency and various duplications covering parts of the region lost in the deficiency. The results may have a bearing on the problem of position effect.

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ORIGIN OF THE DEFICIENCY

Wild-type males from the "Oregon" stock were treated with a heavy dose of X-rays and crossed to females having attached X chromosomes ($\widehat{XX}$). In the offspring of this cross a single male was found (February 1931) which had slightly rough eyes, and short, parallel-sided, and somewhat truncate wings. This aberrant male was crossed to unrelated $\widehat{XX}$ females; all the male offspring exhibited the characteristics of the father. The mutant males were then crossed to wild-type females; the F_1 generation consisted of normal flies. In F_2 slightly less than one-half of the males showed the mutant characters. It was concluded that the new mutant is a sex-linked recessive. Further tests showed that the new gene is an allelomorph of the previously known sex-linked recessive small-wing (sl^1, located at 54.2 in the X chromosome, see MORGAN, BRIDGES, STURTEVANT, 1925). The new mutant is, therefore, called small-wing-2 (sl^2). The external effects of sl^2 are similar, though perhaps slightly more extreme, than those of sl^1.

An attempt to establish a stock homozygous for sl^2 failed, the chromosome carrying sl^2 being lethal when present in two doses in females. This was unexpected since sl^1 is equally viable in females and in males. Further tests showed, however, that the lethal effect of the chromosome carrying sl^2 is not associated with the gene sl^2 itself, but with another locus in the

same chromosome. The locus responsible for the lethal effect is that of the gene bobbed (*bb*, 70.0 in the X chromosome). The presence of a bobbed-allelomorph in the chromosome carrying sl^2 was ascertained by the following test. The sl^2 males were crossed to females homozygous for bobbed. The results are shown in table 1. Females carrying a sl^2 X chromosome and a *bb* X chromosome are extreme bobbed in appearance. In fact, they

TABLE 1

sl^2bb^{def} ♂ × *bb* ♀.

EXTREME BOBBED ♀	WILD-TYPE ♀	EXTREME BOBBED ♂	WILD-TYPE ♂
46	3	2	138

are considerably more extreme than females homozygous for *bb*. It follows that the chromosome carrying sl^2 contains an allelomorph of *bb*. For reasons to be presented below this new allelomorph of *bb* is called bobbed-deficiency (bb^{def}). The X-ray treatment induced, therefore, two independent mutations in the same chromosome, namely sl^2 and bb^{def}. The lethal effect of the sl^2-bb^{def} chromosome is to be ascribed to bb^{def} rather than to sl^2. Indeed, lethal allelomorphs of *bb* are known (bobbed-lethal, bb^l, see MORGAN, STURTEVANT and BRIDGES 1927, STERN 1929a).

The non-appearance of the bobbed-characteristics (short bristles, late hatching from the pupae, sometimes also disarrangement of the abdominal tergites), as well as the viability of the bb^{def} males, is to be expected. STERN (1925, 1927, 1929a) has shown that bobbed is the only known sex-linked gene having a wild-type allelomorph in the Y chromosome. The effect of bb^{def} in the males is usually suppressed by the Y chromosome. Only in males having no Y chromosome (XO males), or in males having a bobbed-allelomorph in the Y chromosome, can the bobbed characters be manifested.

In the offspring of the cross shown in table 1 there were three wild-type females and two extreme bobbed males. These classes are due to non-disjunction of the X and Y chromosomes in the males carrying the sl^2–bb^{def} chromosome. Such males occasionally produce spermatozoa containing both the X and the Y chromosomes, and spermatozoa containing neither of these chromosomes. The first kind of spermatozoa gives rise to XXY females. In such females the effect of bb^{def} is suppressed by the wild-type allelomorph of *bb* in the Y chromosome, and such females are wild-type in appearance. The second kind of spermatozoa produces XO males which are extreme bobbed (XO males manifest bobbed in a more extreme form than females homozygous for bobbed, STERN 1927). Non-disjunction of the X-Y pair of chromosomes occasionally takes place in normal males (STERN 1929b), but its frequency is very low. The frequency observed in

our experiment (2.6 percent, table 1) is higher than normal. It seems that the presence of the sl^2–bb^{def} chromosome increases the frequency of non-disjunction in males.

REDUCTION OF CROSSING OVER PRODUCED BY THE sl^2–bb^{def} CHROMOSOME

Small-wing-2 males were crossed to females homozygous for the combination of the sex-linked recessives known as "X-ple" (*sc ec cv* ct^6 *v* g^2 *f*). The F_1 females were crossed to white males (white is a sex-linked recessive). The results are presented in table 2. The calculated frequencies of crossing over are shown in table 3. The standard frequencies of crossing over in the X chromosome are given in table 3 for comparison (according to BRIDGES and OLBRYCHT 1926, and ANDERSON 1929).

TABLE 2

sc ec cv ct v g 6 7 f / 1 2 3 4 5 *sl* *bb-def* ♀ × *w*♂.

MALES ONLY			
0—*sc ec cv ct v g f*	927	6—*f*	1
0—*sl*	1280	7—*sc ec cv ct v g*	1
1—*sc sl*	5	7—*sl f*	1
1—*ec cv ct v g f*	9	3, 6—*sc ec cv f*	1
2—*sc ec sl*	3	3, 7—*ct v g*	1
2—*cv ct v g f*	2	4, 5—*v sl*	1
3—*sc ec cv sl*	2	4, 6—*sc ec cv ct f*	4
3—*ct v g f*	3	4, 6—*v g sl*	1
4—*sc ec cv ct sl*	6	5, 6—*g sl*	1
4—*v g f*	11	5, 7—*g*	1
5—*sc ec cv ct v sl*	7	non-disjunction—*w*	46
5—*g f*	6	Total	2320

TABLE 3

Frequency of crossing over in females heterozygous for the X chromosome carrying bb^{def}.

INTERVALS	*sc-ec*	*ec-cv*	*cv-*ct^6	ct^6*-v*	*v-*g^2	g^2*-*sl^2	sl^2*-f*
bb^{def}	0.6	0.2	0.3	1.0	0.7	0.4	0.2
Standard	6.8	9.7	8.4	14.8	11.2	11.3	
Difference	−6.2	−9.5	−8.1	−13.8	−10.5	−10.7	

The very strong reduction of the frequency of crossing over in the chromosome carrying sl^2 and bb^{def} is obvious. The following experiments were undertaken in order to determine the frequency of crossing over to the right of the locus of *f*; the intervals lying to the right of *f* were not followed in the experiment described above. Small-wing-2 bb^{def} males were crossed

to carnation females (the gene carnation, *cr*, lies at about 8 units to the right of *f*), and the F_1 females were outcrossed to *w* males. Table 4 shows the results. In the presence of bb^{def} the frequency of crossing over between

TABLE 4

$\frac{sl^2 \quad bb^{def}}{cr}$ ♀ × *w*♂ (males only).

sl	*cr*	*sl² cr*	WILD-TYPE	*w*	TOTAL
849	1282	12	6	35	2184

sl^2 and *cr* is 0.9 percent instead of about 10 percent. In another experiment sl^2 bb^{def} males were crossed to *cr* females, and the F_1 females were outcrossed to *bb* Y^{bb} males (Y^{bb} is an allelomorph of *bb* lying in the Y chromosome; males having *bb* in the X and Y^{bb} in the Y chromosome manifest the characters of bobbed). The results are shown in table 5. The frequencies of crossing over in the sl^2–*cr* and the *cr*–bb^{def} intervals are 0.5 percent and 0.9 percent respectively. Since *sc* and *bb* are located in opposite ends of the X chromosome, the data presented in tables 2 to 5 show that crossing over is strongly reduced throughout the entire X chromosome.

TABLE 5

$\frac{sl^2 \quad bb^{def}}{cr}$ ♀ × *bb* Y^{bb}♂ (*males only*).

sl^2 *bb*	*cr*	sl^2 *cr*	*bb*	sl^2	*cr bb*	EXTREME-*bb*	TOTAL
1385	1861	9	8	20	11	36	3330

A considerable number of flies due to non-disjunction of the X and Y chromosomes appear in the offspring of females heterozygous for sl^2 and bb^{def} (*w* flies in tables 2 and 4, extreme-bobbed flies in table 5). The frequency of non-disjunctional flies varies from 1.1 percent to 2 percent in the different experiments. The normal frequency of non-disjunctional gametes is about 1:1200 (MORGAN, BRIDGES, STURTEVANT 1925). It is concluded that the sl^2–bb^{def} chromosome is responsible not only for the reduction of crossing over but also for the increase in the frequency of non-disjunction.

SEPARATION OF BOBBED-DEFICIENCY FROM SMALL-WING-2

Since crossing over occurs between sl^2 and bb^{def}, these genes can be separated from each other. The sl^2 *f* male shown in table 2, and the sl^2 *cr* males shown in table 4, carry the left part of the original sl^2–bb^{def} chromosome but, presumably, do not carry its right part (containing bb^{def}). Conversely, the wild-type males shown in table 4 carry the right part of the

original chromosome but do not carry its left part (containing sl^2). Homozygous sl^2 *f* and sl^2 *cr* stocks were established without difficulty. An attempt to establish a stock homozygous for bb^{def}, but not carrying sl^2, failed. The lethal effect of the sl^2–bb^{def} chromosome in double dose in females is clearly due to bb^{def} and not to sl^2.

The following experiment shows that sl^2 without bb^{def} does not influence the frequency of crossing over in the X chromosome. Crosses were made of sl^2 *cr* males to *y cv v f* females. The F_1 females were crossed to white-eyed males. Among the 1573 males counted in the next generation there were only two white ones; the frequency of non-disjunction in the presence of sl^2, without bb^{def}, is not significantly different from normal (see above). Table 6 shows the frequency of crossing over observed in this experiment. The frequencies observed are not significantly different from the standard values.

TABLE 6

$\frac{y\ cv\ v\ \ f}{sl\ \ cr}$ ♀ × *w*♂ (*males only*).

INTERVAL	*y-cv*	*cv-v*	*v-sl*	*sl-f*	*f-cr*
Frequency	12.0	20.7	19.6	2.4	8.2

Males carrying bb^{def} without sl^2, as well as females heterozygous for bb^{def}, do not differ from the wild-type in appearance, but their viability and fertility seem to be somewhat below that of the wild-type. The presence of bb^{def} may be detected phenotypically by making flies heterozygous for other allelomorphs of *bb*. Thus, females of the structure bb/bb^{def} are extreme bobbed (table 1). Likewise, males of the structure bb^{def}/Y^{bb} show the bobbed characteristics in a more extreme form than males of the constitution bb^{t}/Y^{bb}, but much less extreme than XO males carrying *bb*. The allelomorph bb^{def} seems, therefore, to be completely recessive to the wild-type allelomorphs located in either X or in Y chromosomes; in compounds with other *bb* allelomorphs it behaves as the most extreme allelomorph of *bb* thus far known.

PROBABLE NATURE OF bb^{def}

Point-mutations do not, as a rule, affect the frequency of either crossing over or non-disjunction of the chromosomes in which they lie. On the contrary, chromosomal aberrations, such as translocations, inversions, deficiencies, and duplications, frequently affect both crossing over and disjunction. The behavior of bb^{def} suggests, therefore, that not a point-mutation, or at least not only a point-mutation, but some chromosome-aberration is responsible for its appearance and the manner of its action. Tests

were made for the presence of a translocation associated with bb^{def}. The results were clearly negative for all the chromosomes. The assumption that an inversion arose in the X chromosome simultaneously with bb^{def} is improbable, since the presence of bb^{def} allows some single crossing over to take place in every interval studied; this is not the case in any known inversions.

The mutation from wild-type to bb^{def} is best interpreted as due to a deficiency, that is, to the loss of a section of the X chromosome carrying the normal allelomorph of bobbed. According to MOHR (1923, 1927, 1929) deficiencies behave as the most extreme allelomorphs of the known recessives whose loci are included in the deficient region. This is the case with bb^{def} (see above). Like all the other known deficiencies bb^{def} is lethal when homozygous. All known deficiencies eliminate crossing over in the sections

FIGURE 1.—Chromosomes of females heterozygous for bobbed-deficiency. X, the normal X chromosome; D, X chromosomes carrying the deficiency.

involved (BRIDGES 1917, MOHR 1923, 1927), but the longest ones also decrease the frequency of crossing over in the sections of the chromosome adjacent to the deficient section (LI and BRIDGES 1929, MORGAN, BRIDGES and SCHULTZ 1931). The cytological findings (see below) prove that bb^{def} is actually a deficiency.

CYTOLOGY OF BOBBED-DEFICIENCY

The phenomenon of deficiency was discovered and studied in Drosophila by BRIDGES (1917, 1919) and by MOHR (1919, 1923, 1927, 1929). BRIDGES (1917) defined the term "deficiency" to mean "the loss or inactivation of an entire, definite, and measurable section of genes and framework of a chromosome." The deficiencies studied by BRIDGES and MOHR were short

and invisible cytologically. The assumption that the deficient genes were actually lost from the chromosome was inferred from the genetic data. For this reason BRIDGES admitted the possibility that the deficient genes were not physically lost but merely inactivated.

Some of the more recently described deficiencies have proved to be sufficiently extensive to produce cytologically visible changes in the chromosomes. To this group belong the "v–o" deficiency in mice (PAINTER 1927), several deficiencies in the X chromosome of Drosophila (L. V. MORGAN in MORGAN, BRIDGES and STURTEVANT 1928, PAINTER and MULLER 1929, MULLER and PAINTER 1932, DOBZHANSKY 1932a, b), and in *Zea mays* (MCCLINTOCK 1931). PAINTER (1927) proposed the term "deletion" for cases in which the loss of genes is discoverable both genetically and cytologically. Evidently, most if not all of the deficiencies studied by BRIDGES and MOHR are deletions in PAINTER'S sense. The existence of two terms for precisely the same phenomenon seems unnecessary.

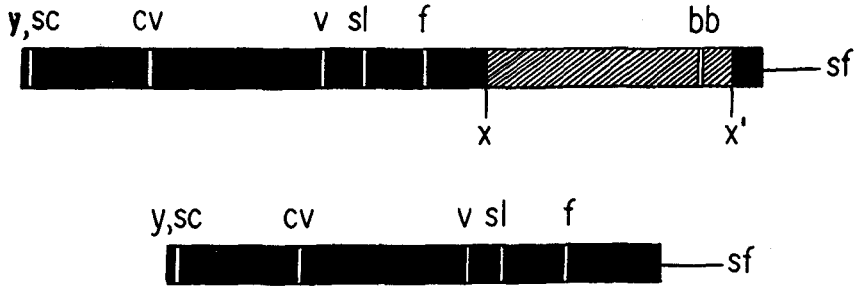

FIGURE 2.—The normal X chromosome (above), and the X chromosome carrying bobbed-deficiency (below). X and X¹, the points at which the chromosome was broken. The shaded part, the section which is lost in the deficiency. *sf*, the spindle fibre. Other letters, location of various genes.

Bobbed-deficiency behaves genetically like the deficiencies studied by BRIDGES and MOHR. The deficiency-nature of the allelomorph bb^{def} was inferred from the genetic data presented above. This deficiency involves the locus of a single known gene, namely that of bobbed. Nevertheless, it is clearly visible cytologically.

Wild-type females were crossed to sl^2 bb^{def} males. All the females in the offspring of this cross should be heterozygous for bb^{def} (barring the possibility of primary non-disjunction). Female larvae were selected, and their nerve-ganglia were fixed in Navashin's solution. Several chromosome-plates were found in this material. Some of them are reproduced in figure 1. In each plate one may see two X chromosomes which are unequal in length. The longer chromosome (X) is presumably the normal X chromosome. The shorter one (D) is the X chromosome deficient for bobbed. The

shorter chromosome is approximately two-thirds the length of the longer chromosome present in the same plate. Hence, in bobbed-deficiency, about one-third of the X chromosome is lost.

Figure 2 represents schematically the structure of the X chromosome in bobbed-deficiency, and also an interpretation of the mode of its origin. The normal X chromosome (figure 2A) was broken in two places (x and x^1), and the section lying between x and x^1 was lost. The sections lying to the left and to the right of x and x^1 respectively became united, producing the bobbed-deficiency chromosome (figure 2B). An alternative assumption is that the X chromosome was broken at a single locus (that is, at x), and that the section lying to the right of x was lost. Such an assumption seems, however, improbable since the spindle-fibre is known to be attached to the right end of the X chromosome. The behavior of translocations and deficiencies in Drosophila indicates that a chromosome which has lost its spindle-fibre attachment does not behave normally in mitosis, and is therefore eliminated.

DUPLICATIONS FOR BOBBED

Several duplications for various sections of the X chromosome were found in the progeny of males treated with X-rays. The origin of these duplications is mostly due to a loss ("deletion," PAINTER and MULLER, 1929) of the middle region of the chromosome, followed by a reunion of the end regions. The resulting chromosomes contain, consequently, only a part of the genes normally located in the X, and consist of two parts corresponding to the left and the right ends of the normal X respectively. Most of the duplications are so small that their addition to the chromosomal compliment of males (respectively, females) does not upset the sex balance of the resulting hyperploid individuals. Thus, a duplication female carries two normal X's plus the duplication, that is, a fragment of a third X. Similarly, a duplication male carries one normal X, one Y chromosome, and the duplication.

Six different duplications, covering small portions of the left end of the X chromosome, were selected for the purposes of the present study. They are denoted as "Duplication 101" (described in DOBZHANSKY 1932a), "Duplication 106," 107, 118, 136 (DOBZHANSKY 1932b), and "Duplication 135" (undescribed). The methods used for determination of the loci present in the duplications were outlined in the papers just referred to, and are, therefore, only briefly reviewed here. Individuals are obtained which are homozygous for definite recessive sex-linked genes, and which carry the duplication in question. If the duplication contains the dominant wild-type allelomorphs of the respective recessives, the duplication-carrying individuals fail to manifest the characteristics of the recessives (the duplications contain only wild-type allelomorphs of the sex-linked recessives since

the individuals used for X-rays treatment are usually wild-type males). It was found by this method that duplications 101, 106, 107, 118, and 135 carry the loci of yellow (*y*), scute (*sc*) and silver (*sv*). The duplication 136 carries in addition the loci of kurz (*kz*), broad (*br*) and prune (*pn*).

For the determination of the presence of the locus for bobbed, a slightly different method is used. Females having attached X chromosomes ($\widehat{XX}$), and homozygous for certain sex-linked recessives (the genetic structure of such females is represented in the upper left corner of figure 3), are crossed to bb/Y^{bb} males (upper right corner of figure 3; Y^{bb} is an allelomorph of bobbed located in the Y chromosome). Females produced in the offspring of this cross are $\widehat{XX}/Y^{bb}$ (figure 3). Such females are crossed to males carrying the recessives *y*, *sc*, *cv*, *f* and bb^{l} in their normal X's, and carrying the duplication to be tested. In the next generation two kinds of males are obtained (lower line in figure 3). Both kinds carry bb^{l} and Y^{bb}, but one kind carries the duplication, and the other is free from it. If the duplication does not contain the locus of bobbed, all males show bobbed in a rather extreme form. If the wild-type allelomorph of bobbed is present in the duplication, the resulting male is non-bobbed. The presence of duplications in a given male is recognized by the suppression of the effects of *y* and *sc*. The results of testing the duplications for the presence of the

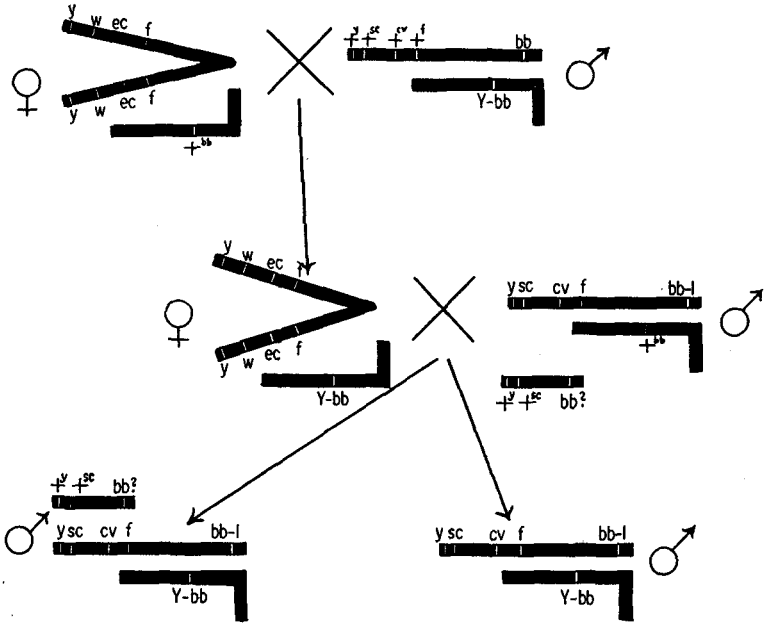

FIGURE 3.—The experimental procedure for testing for the presence of the wild-type allelomorph of bobbed in the duplications. The >shaped chromosome, the attached X chromosomes ($\widehat{XX}$); the rod-shaped chromosome, the X chromosome; the hook-shaped chromosome, the Y chromosome; the short rod-shaped chromosome, the duplication.

locus for bobbed are shown in table 7. One may conclude that duplication 106 does not carry the locus of bobbed, while all other duplications do carry that locus.

TABLE 7

$y^2 w^a$ ec f $\widehat{XX}$ / Y^{bb} ♀ × y sc cv f bb^l / Y / duplication ♂.

DUPLICATION	$y^2 w^a$ ec f ♀	w^a ec f ♀	y sc cv f bb ♂	cv f bb ♂	cv f ♂
101	274	349	326	..	241
106	190	316	205	67	..
107	96	222	165	..	128
118	115	259	150	..	116
135*	161	314	168	..	134
136*	75	146	81	..	59

* Duplications 135 and 136 carry y^2 (an allelomorph of y) in the fragment. For testing them $\widehat{XX}$ yellow females were used instead of the $\widehat{XX}$ y^2 w^a ec f ones.

A different method of testing for the presence of the bobbed-locus in the duplications consists in using females of the structure bb/bb^l. Such females are, in the absence of duplications, extreme bobbed in appearance (figure 4). If a duplication containing the wild-type allelomorph of bb is added to the chromosomes of such females (the resulting constitution is bb/bb^l/duplication, figure 4), the flies are wild-type. The duplications were also tested by this method; the results were the same as those presented above.

It should be kept in mind, that both methods of testing for the presence of the locus for bobbed in duplications have the following limitation. It is assumed that the duplications may carry either the wild-type allelomorph of bobbed, or not carry the bobbed locus at all. This assumption is based on the fact that the duplications are obtained by treating wild-type (that is, non-bobbed) males with X-rays. However, the X-ray treatment may induce a mutation to bobbed in the duplicating fragment itself. It is easy to see that in such a case the tests may lead to the conclusion that the duplications do not carry bobbed at all. The negative results of the tests are thus not entirely conclusive, while the positive results are more convincing.

INTERACTION OF BOBBED-DEFICIENCY WITH BOBBED-DUPLICATIONS

Bobbed-deficiency behaves as a recessive. Its external effects are suppressed by the presence of a single wild-type allelomorph of bobbed. Thus, females of the constitution $bb^{def}/+^{bb}$, and males of the constitution bb^{def}/Y^+ (figures 4 and 5), are wild-type. Five of the six duplications described above seem to carry wild-type allelomorphs of bobbed. It is, consequently, justifiable to expect that the presence of these duplications will

suppress the effects of bobbed-deficiency. The experiments show that this expectation is not realised.

Males of the constitution bb^{def}/Y^{bb}/duplication were secured. The experiments were so arranged, that males of the constitution bb^{def}/Y^{bb} were obtained in the same cultures (the experimental procedure applied for getting the bb^{def}/Y^{bb}/duplication and the bb^{def}/Y^{bb} males is similar to that diagrammed in figure 3. The difference consists in using *y* sl^2 bb^{def}/Y^+ males instead of the *y sc cv f* bb^l males shown in figure 3). The two kinds of males are exactly alike, except for the presence of the duplication. The inspection of the bb^{def}/y^{bb}/duplication males has shown that they are not wild-type, as expected, but are more or less extreme bobbed. Their bristles are short and slender, the abdominal tergites are frequently disarranged, the viability is low. In the absence of bobbed-deficiency the duplications do not produce these characteristics. It seems clear that the wild-type allelomorph of bobbed lying in the duplications fails to suppress bobbed-deficiency.

Some measurements were undertaken for the purpose of obtaining more precise information on the degree of suppression of bb^{def} by various duplications. Bristles were measured in flies of the constitution bb^{def}/Y^{bb}/duplication and bb^{def}/Y^{bb}. Flies were macerated in a solution of KOH. After washing in water, the flies were transferred in glycerine for clearing. The heads and the thoracal parts of the cleared flies were then isolated, arranged in rows in drops of glycerine on slides, covered with thick cover slips, and flattened as much as possible by means of pressing on the cover slips. The length of the inner vertical, the posterior dorsocentral, and the posterior scutellar bristles was then measured in terms of the units of an eyepiece-micrometer (1 unit being equal to 8.9 mikra). Only one bristle of each kind was measured in each fly. By the "length of the bristle" is meant the distance between the free end of the bristle and its insertion into the theca, irrespective of whether the bristle is absolutely straight or slightly curved.

The results of the measurements are presented in tables 8, 9, and 10. The graphs marked "duplication" indicate the length of the bristles in bb^{def}/Y^{bb}/duplication flies; the graphs marked "control" give similar data for the bb^{def}/Y^{bb} flies from the same cultures. The data for the bb^{def}/Y^+ (wild-type) flies serve as the standard of comparison. For each of the forms studied the mean value (M), its mean error (m), the standard deviation (σ), coefficient of variation (C), the limits of variation (Lim), and the number of flies measured (n) are given.

The bristles in the bb^{def}/Y^{bb}/duplication males are in no case as long as they are in wild-type (bb^{def}/Y^+) males. The differences are statistically significant, with a single exception of the dorsocentral bristles in duplica-

TABLE 8.

Length of the inner vertical bristle in bb^{def}/Y^{bb}/ duplication and in bb^{def}/Y^{bb} males.

	$M \pm m$	$\sigma = \pm$	C	*Lim*	n
bb^{def}/Y^{+} (wild-type)	30.87±0.20	1.78	5.8	25–35	81
Duplication 101	24.71±0.22	1.79	7.3	21–35	68
Control 101	20.00±0.40	1.94	9.7	16–24	24
Duplication 106	21.06±0.32	2.36	11.2	16–25	54
Control 106	20.67±0.57	2.82	13.6	17–28	24
Duplication 107	27.29±0.22	1.98	7.3	22–31	81
Control 107	21.14±0.50	2.52	11.8	17–25	25
Duplication 118	21.48±0.27	1.72	8.0	18–27	41
Control 118	21.14±0.45	2.24	10.6	15–24	25
Duplication 135	27.62±0.43	1.74	6.3	25–30	16
Control 135	23.03±0.35	1.36	5.9	21–25	15
Duplication 136	20.68±0.30	1.83	8.9	18–25	37

TABLE 9

Length of the posterior dorsocentral bristle in bb^{def}/Y^{bb}/ duplication and in bb^{def}/Y^{bb} males.

	$M \pm m$	$\sigma = \pm$	C	*Lim*	n
bb^{def}/Y^{+} (wild-type)	36.84±0.25	2.30	6.3	30–40	81
Duplication 101	32.26±0.26	2.13	6.6	26–38	68
Control 101	25.83±0.47	2.29	8.9	22–30	24
Duplication 106	27.91±0.40	2.94	10.5	23–33	54
Control 106	27.25±0.74	3.60	13.2	23–36	24
Duplication 107	34.62±0.24	2.18	6.3	30–40	81
Control 107	28.18±0.39	1.94	6.9	24–32	25
Duplication 118	28.02±0.31	1.96	7.0	24–32	41
Control 118	28.18±0.42	2.08	7.4	22–30	25
Duplication 135	35.75±0.39	1.56	4.4	32–38	16
Control 135	29.43±0.37	1.42	4.8	27–32	15
Duplication 136	26.68±0.23	1.40	5.2	24–30	37

TABLE 10

Length of the posterior scutellar bristle in bb^{def}/Y^{bb}/duplication and in bb^{def}/Y^{bb} males.

	$M \pm m$	$\sigma = \pm$	C	*Lim*	n
bb^{def}/Y^{+} (wild-type)	47.91±0.22	1.95	4.1	43–52	81
Duplication 101	38.62±0.21	1.71	4.4	35–42	68
Control 101	33.42±0.67	3.31	9.9	29–40	24
Duplication 106	33.87±0.42	3.06	9.0	28–39	54
Control 106	33.92±0.98	4.80	14.2	27–42	24
Duplication 107	43.34±0.23	2.10	4.8	38–48	81
Control 107	35.62±0.80	4.02	11.3	27–44	25
Duplication 118	35.08±0.34	2.18	6.2	30–40	41
Control 118	35.70±0.51	2.54	7.1	27–38	25
Duplication 135	44.88±0.44	1.76	3.9	42–49	16
Control 135	36.23±0.42	1.76	4.9	34–48	15
Duplication 136	33.00±0.31	1.86	5.6	30–37	37

tion 135. It follows that none of the duplications studied suppress completely the effects of bobbed-deficiency. On the other hand, the degree of suppression produced by different duplications is variable. Duplication 135 comes closest to producing a complete suppression. Duplication 107 follows next. Duplication 101 has, in the presence of bb^{def}, bristles roughly intermediate in length between those observed in wild-type and in bb^{def}/Y^{bb} males. Finally, duplications 118 and 136 behave very nearly as duplication 106, in spite of the fact that the two former carry the wild-type allelomorph of bobbed, and the latter has no bobbed locus at all.

The effect of duplications on bb^{def} was also tested by a different method. Females of the constitution *ClB/y* sl^2 bb^{def} were crossed to *y* bb/Y^+/duplication males. Some of the non-bar females obtained in the next generation were yellow, and others were non-yellow. The yellow females have the constitution *y* sl^2 bb^{def}/bb, and the non-yellow ones the constitution *y* sl^2 bb^{def}/bb/duplication. If the duplications suppress the effects of bb^{def}, the *y* sl^2 bb^{def}/bb/duplication females should be non-bobbed. In fact they are more or less distinctly bobbed. The behavior of the different duplications in females is similar to their behavior in males. Thus, duplications 107 and 135 produce a fairly strong, though incomplete, suppression of bb^{def} in females, while duplication 118 fails to produce marked effects.

The facts presented above apparently leave no escape from the conclusion that the behavior of the wild-type allelomorph of bobbed lying in duplicating fragments is different from the behavior of the same allelomorph lying in the complete X or Y chromosomes. It is worth while to consider here some sources of error, which might conceivably invalidate the above conclusion. One may suppose, for instance, that the presence of the duplications, by altering the genic balance, is *per se* responsible for a decrease (or an increase) of the length of the bristles. This supposition is invalid, since in the absence of bobbed, the duplications (perhaps with the single exception of duplication 136, which makes the bristles thicker and shorter), do not seem to affect the size of the bristles. It is, of course, possible that a very slight effect on the bristle size is produced by the duplications. It remains, however, to be explained why different duplications behave so differently in compounds with bb^l on one hand and in compounds with bb^{def} on the other.

One may also suppose that the allelomorph Y^+ (that is, the wild-type allelomorph of bobbed lying in the Y chromosome) is more effective in suppressing bb^{def} in males than is $+^{bb}$ (that is, the wild-type allelomorph of bobbed lying in the X chromosome). If this were so, then the difference in the bristle length which exists between the bb^{def}/Y^+ and the bb^{def}/Y^{bb}/ duplication males might be attributed to the higher efficiency of Y^+ as compared with $+^{bb}$. This supposition is contrary to the following two

facts: (a) different duplications behave differently in the compound bb^{def}/Y^{bb}/duplication; (b) the behavior of the duplications is similar in both sexes.

DISCUSSION OF THE RESULTS

The inert region of the X chromosome

Bobbed-deficiency represents a loss of about one-third of the whole X chromosome. A loss of such a long section of a chromosome might be expected to produce a considerable upset of the genic balance, and, consequently, a somatic effect. This is not the case with bobbed-deficiency. As shown above, bb^{def} is lethal when homozygous, but has little, if any, effect on the heterozygotes. Females of the constitution $bb^{def}/+$, and males

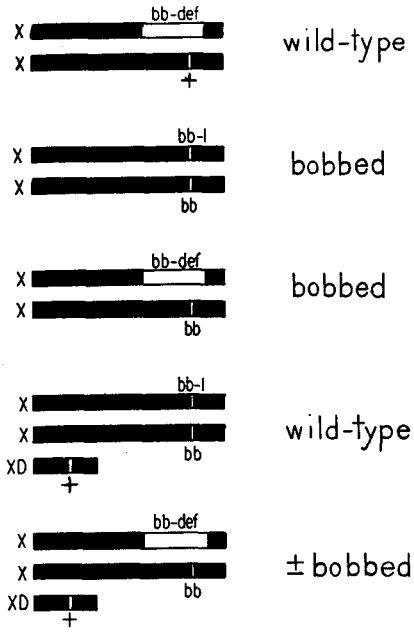

FIGURE 4.—The interaction of the different bobbed allelomorphs in females, X, the X chromosome; XD, the duplication; the white area, the section which is lost in bobbed-deficiency.

of the constitution bb^{def}/Y^{+} have a somewhat lower viability than wild-type flies, but they are seemingly completely normal in appearance. Deficiencies which are too short to be visible cytologically frequently produce striking dominant somatic effects (MOHR 1923, 1929), and relatively very short deficiencies may be completely lethal in heterozygotes (see, for example, DOBZHANSKY 1930 and 1931). This indicates that the region which is lost in bb^{def} is less important for development than regions of similar lengths in other chromosomes of Drosophila.

PAINTER (1931a, b), MULLER and PAINTER (1932), and DOBZHANSKY (1932b) have shown by combined genetical and cytological studies that

the right one-half or one-third of the X chromosome is made up of a region in which only one gene, namely bobbed, is known to be located. Furthermore, very little, if any, crossing over takes place in this region. PAINTER called this region the "inert region," implying that the number of functional genes per unit of distance is very small in this region. The behavior of bobbed-deficiency constitutes new and fairly conclusive evidence in favor of this view.

The material contained in the inert region of the X chromosome is supposed to be homologous to a section of the Y chromosome. The Y chromosome is also known to be composed of predominantly inert material. As a matter of fact, this assumption seems to have been warranted on the basis of facts which were discovered before anything was known regarding the

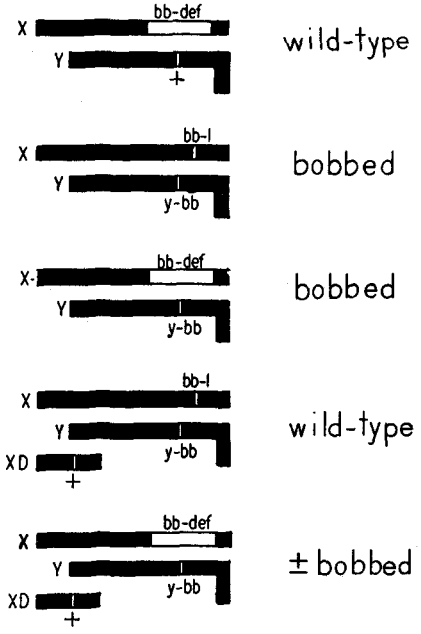

FIGURE 5.—The interaction of the different bobbed allelomorphs in males. Y, the Y chromosome. The significance of other letters is the same as in figure 4.

existence of an inert region in the X chromosome. METZ (1926) in his studies on the spermatogenesis of Drosophila has shown that the X and the Y chromosomes do not pair at synaptic stages along their entire lengths, but that only a part of the X undergoes pairing with the Y, the rest of the X remaining unpaired. This indicates that a part of the X is homologous with a part of the Y chromosome.

The behavior of bobbed-deficiency provides further confirmation of the above assumption. Bobbed-deficiency males are normal phenotypically. If some of the genes located in the region of the X which is lost in

bb^{def} have no allelomorphs in the Y chromosome, then bb^{def} males should not carry these genes at all. It is known, however, that all the known deficiencies so far studied in Drosophila are invariably lethal when homozygous. The data of LI (1926) show, furthermore, that individuals homozygous for deficiencies die in very early stages of embryonic development. These data, in the opinion of the present writers, indicate that most, if not all of the genes in Drosophila are essential for development, irrespective of whether the known mutations of these genes produce alterations of "superficial" or "fundamental" characters. From this point of view it is improbable that a male which has lost X chromosome genes having no allelomorphs in the Y chromosome would be at all viable, not to speak of being phenotypically normal.

The cytological findings in bobbed-deficiency serve to establish the minimum length of the inert region of the X chromosome. The length of this region is not less than one-third of the length of the X.

Behavior of the wild-type allelomorph of bobbed in duplications

Bobbed-deficiency is recessive to the wild-type allelomorph of bobbed, provided the latter lies in a normal, unbroken X or Y chromosome. Thus $bb^{def}/+^{bb}$ females and bb^{def}/Y^{+} males are wild-type. The situation is different if the wild-type allelomorph of bobbed lies in a fragment of an X chromosome. Five duplications carrying the locus of bobbed were studied. According to their origin they should carry the wild-type allelomorph of bobbed. None of these duplications suppresses the effects of bb^{def}. Individuals of the constitution bb^{def}/Y^{bb}/duplication and bb^{def}/bb/duplication are more or less clearly bobbed. Three possible explanations of this phenomenon may be discussed here.

First explanation

The observed facts may indicate that: (a) the development of the wild-type characteristics depends upon the presence of a definite amount of the substance located in the so-called "inert" region of the X, and in the corresponding region of the Y chromosome, rather than upon the action of a specific locus located in these regions; (b) the known allelomorphs of bobbed (bb^{def}, bb^{l}, bb, Y^{bb}) represent losses of varying amount of this substance, which, for the purposes of the present discussion we may call "the bobbed-substance"; (c) the smaller the amount of the "bobbed-substance" present in the germ plasm, the more extreme become the characteristics of bobbed in the adult fly.

None of the duplications studied include the entire inert region of the X chromosome (DOBZHANSKY 1932b). Hence, none of them carries the

amount of the "bobbed-substance" present in the normal X. The duplications include, however, enough of the "bobbed-substance" to produce the wild-type condition in the combinations bb^l/Y^{bb}/duplication and bb^l/bb/duplication. Since bb^{def} represents a longer deficiency than bb^l, the presence of the duplications is not sufficient to produce the wild-type condition in bb^{def}/Y^{bb}/duplication and bb^{def}/bb/duplication flies (figures 4 and 5).

STERN (1929a) has shown that accumulation of the bobbed allelomorphs in the germ plasm results in a gradual approach toward the wild-type condition in the phenotype. Thus, according to STERN, individuals of the constitution bb^l/bb^l are inviable, bb^l/bb are extreme bobbed, $bb^l/bb^l/Y^{bb}$ are less extreme, $bb^l/bb^l/Y^{bb}/Y^{bb}$ are still less extreme, $bb^l/bb/Y^{bb}$ are close to wild-type, and $bb^l/bb/Y^{bb}/Y^{bb}$ are not distinguishable from wild-type. It is easy to see that these results of STERN harmonize perfectly with the interpretation that all bobbed allelomorphs are deficiencies.

STERN discovered, however, another fact which is contradictory to our first explanation. The extreme bobbed allelomorph, known as bobbed-lethal (bb^l), was repeatedly observed to revert to a less extreme allelomorph (bb), and directly to wild-type. This fact is difficult to reconcile with the assumption that bb^l is a deficiency.

Second explanation

The wild-type allelomorph of bobbed is one of the frequently mutating loci. Spontaneous mutations from wild-type to various bobbed allelomorphs are rather common. Though the frequency of mutations at the bobbed locus under the influence of X-rays is unknown, it is not unreasonable to suppose that this frequency is high. The origin of the duplications is due to the breakages caused by X-ray treatment. It is, then, possible that mutations from wild-type to weak allelomorphs of bobbed were induced in the duplications at the time of their origin. This would explain the behavior of the duplications in combinations with bb^{def}.

This explanation meets with a difficulty, for every one of the five duplications studied needs to be supposed to carry a bobbed allelomorph induced by X-rays. Even if the mutation rate of the bobbed locus is much higher than that for any other known gene, it is very improbable that five duplications would by chance carry such mutations. If the phenomena observed are to be accounted for by mutations at the bobbed locus, an additional assumption is necessary, namely that the occurrence of a breakage in the chromosome strongly increases the probability of mutations taking place in the same chromosome. Such a possibility is, of course, not to be disregarded on *a priori* grounds. There exist, indeed, some facts which argue in favor of such possibility. Translocations very frequently carry lethals,

or mutations producing visible effects, the loci of which are associated with the loci at which the chromosomes were broken (MULLER and ALTENBURG 1930, DOBZHANSKY 1930, 1932c). STERN and OGURA (1931) observed mutations at the bobbed locus which arose simultaneously with translocations involving the X and the Y chromosomes.

Third explanation

The effect of the wild-type allelomorph of bobbed on development may depend upon its structure as well as upon its position in the chromosome. The loss of the middle part of the X chromosome involves a removal of the material normally located in the vicinity of bobbed, and establishing an association between the locus of bobbed and other loci lying normally far from bobbed. The behavior of the wild-type allelomorph of bobbed in the duplications may, thus, be accounted for by "position effect."

The phenomenon of position effect was discovered in Drosophila by STURTEVANT (1925, 1928), who demonstrated that two Bar genes lying in the same chromosome produce a stronger effect than two Bar genes lying in opposite chromosomes. The appearance of "mutations" at the loci of breakages in translocations (see above) may be accounted for by position effect as well as by mutation, and in some cases the explanation by position effect is distinctly preferable to that by mutation (DOBZHANSKY 1932c). The behavior of certain other genes, besides bobbed, in duplications also suggests the existence of a position effect (DOBZHANSKY and STURTEVANT 1932).

The second and the third explanations account equally well for the observed behavior of the wild-type allelomorph of bobbed in the duplications. At the present there seems to be no way for distinguishing between these two explanations experimentally. It is perhaps desirable to point out here that these two explanations may not be mutually exclusive. Mutation represents an alteration of the structure of the gene, and may arise without a breakage taking place in the vicinity of that gene. In case of mutation the alteration is permanent, in the sense that the original condition may be regained only by a reverse mutation. In case of position effect the alteration of the functioning of the gene is due to the removal of other genes normally lying in the neighborhood of the gene in question, or to the association with genes lying normally far from it. Hence, position effect should disappear as soon as the normal order of genes is restored. It is possible, however, that the bonds existing between the adjacent genes in the chromosome are so intimate, that the rupture of these bonds may lead to irreversible alterations in the structure of the gene itself. In such a way a permanent alteration of the structure of the gene (mutation) may be brought about by a change in the position of that gene in the chromosome (position effect).

SUMMARY

1. A deficiency for the sex-linked gene bobbed was found in the progeny of males treated with X-rays. The presence of the deficiency (symbol bb^{def}) decreases the frequency of crossing over, and increases the frequency of non-disjunction of the X chromosomes.

2. The X chromosome carrying the deficiency is about two-thirds as long as the normal X. It follows that the "inert" region makes up at least one-third of the length of the normal X.

3. The deficiency behaves as a recessive to the wild-type allelomorph of bobbed. Thus, $bb^{def}/+^{bb}$ females and bb^{def}/Y^{+} males are wild-type in appearance. In compounds with other allelomorphs of bobbed (bb, Y^{bb}) the deficiency produces an exaggeration of the bobbed characteristics.

4. Five duplications for sections of the X chromosome carrying the locus of bobbed are described. According to their origin these duplications should carry wild-type allelomorphs of bobbed.

5. The wild-type allelomorph of bobbed lying in the duplications fails to suppress the effects of bobbed-deficiency. Three possible explanations of this phenomenon are discussed.

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