DEFICIENCY[1]

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INTRODUCTORY SUMMARY

The general term "deficiency" is used to designate the loss or inactivation of an entire, definite, and measurable section of genes and framework of a chromosome. A case of deficiency in the X chromosome of *Drosophila ampelophila* occurred in September 1914, and has given rise to a whole series of correlated phenomena.[2] The first indication of this deficiency was the occurrence of a female which had failed to inherit from her father his sex-linked dominant mutant "bar",[3] though she inherited in a normal manner his sex-linked recessive mutant "white." This female, when bred, gave only about half as many sons as daughters, the missing sons, as shown by the linkage relations, being those which had received that X which was deficient for bar. This lethal action in-

[1] Contribution from the Zoölogical Laboratory of COLUMBIA UNIVERSITY, and the CARNEGIE INSTITUTION OF WASHINGTON.

[2] A brief account of deficiency was included in "Non-disjunction as proof of the chromosome theory of heredity." Genetics **1**: 1-52, 107-163, Jan.-Mar., 1916. A fuller account was read before the American Society of Naturalists at the meeting held at COLUMBIA UNIVERSITY on December 29, 1916.

[3] For information about the various mutants, and for an explanation of the terms and symbols used, see appendix, p. 456.

dicates that the deficiency mutation involved not simply the bar, but was also a deficiency for one or more genes necessary to the life of the fly. It was next found that the deficiency was extensive enough so that it included the locus for "forked," a recessive mutant whose gene lies in the X chromosome about half a unit from the locus of bar. A deficient-bearing female behaves as though haploid for forked, so that a $\frac{\quad}{f}$ female is forked although forked is a strict recessive. That the region between forked and bar was likewise affected, was demonstrated by the disappearance of crossing over between these loci in females having a deficient X. The deficiency mutation is thus proved not only to have affected a region of adjacent genes but also to have affected the framework of the chromosome on which crossing over must primarily depend. The maximum and the minimum lengths of the deficient region were measured by two genetic methods—by means of haploid tests and by means of linkage. The length of the chromosome as tested by linkage was found to be shorter than normal by an amount corresponding to the length of the deficient region. Unfortunately the stock of deficiency was lost before a cytological examination was made. The fact that the female with one X deficient for bar and the other X carrying bar $\left(\frac{\quad}{B'}\right)$ is an intermediate like the normal bar heterozygote, leads to the conclusion that the intermediate eye shape is due to the broadening action of genes outside the bar locus rather than to a broadening action of the normal allelomorph of bar (b'). Of the two alternative explanations of the nature of deficiency, viz., physical loss and complete inactivation, the loss view is perhaps slightly favored by the evidence. The origin of the dominant mutant bar can not be explained on the "presence and absence" hypothesis as due to the loss of an inhibitor, for the loss of the inhibitor region through deficiency does not give a result comparable to the bar mutation. Sex differentiation was not affected by the occurrence of deficiency; hence sex is determined by specific differentiators which are in some part of the X other than the region from forked to bar. This case of deficiency enables us to establish an identity between the actual localization of certain genes in the X chromosome and their positions as mapped by means of linkage.

THE OCCURRENCE OF THE DEFICIENCY FOR BAR

The exception which led to the discovery of the first case of deficiency occurred (September 25, 1914) among the offspring of an XXY wild-

type female heterozygous for eosin and for vermilion $\left(\frac{w^e}{v}\right)$ which had been outcrossed to a white bar male $\left(\frac{w}{\quad B'}\right)$ of a pure stock (table 1). Bar is a dominant mutant, for which reason all

TABLE 1

The occurrence of the bar-deficient exception among the offspring of a $\frac{w^e}{v}$ ♀ *outcrossed to a white bar.* ♂.

	Regular ♀♀		Regular ♂♂				Exceptions by secondary non-disjunction		Exception by bar-deficiency
			$\frac{w^e}{v}$		$\frac{w^e}{\quad}\vert\frac{v}{\quad}$				
No.	w-w^eB'	B'	w^e	v	w^ev	+	+♀	wB'♂	w-w^e♀
546*	60	59	45	48	14	18	10	10	1

* This culture appeared in table 9, p. 23, of "Non-disjunction as proof of the chromosome theory of heredity," Genetics **1**: Culture 546 is not included in the summary of table 14, appendix.

the daughters were bar,—either white-eosin bar $\left(\frac{w^e}{w}\right)$ or simply bar $\left(\frac{v}{w}\right)$,—except one, which was white-eosin but *not bar* $\left(\frac{w^e}{w}\right)$. That is, *she had inherited the white from her father but had not inherited his bar.* If the genes for all the sex-linked characters are transmitted to daughters by means of a common vehicle, viz., the single X chromosome, then so long as this X is intact and behaves as a unit there should be no chance for him to transmit some of his sex-linked characters without transmitting all of them, as occurred in this case.

The mutation responsible for the bar-deficiency occurred in the germ tract of the white bar male at or close to the maturation division; for he produced many regular daughters but only one with the bar gene deficient.

THE LETHAL ACTION OF DEFICIENCY

The white-eosin exceptional daughter, outcrossed to a wild male, gave (table 2) no bar offspring whatever,—a fresh proof of the deficiency of bar. The sons were of the two expected classes, eosin and white; white was thus again proved to have been transmitted. But the total of the

TABLE 2

The lethal result given by the white-eosin bar-deficient female of culture 546 when outcrossed to a wild male.

Culture No.	Daughters	Sons			
		$\frac{w}{w^e}$ —		$\frac{w}{w^e}$ | —	
		Dies	w^e	w	Dies
593	84	—	37	14	—

sons (51) was only about half the total of the daughters (84), and the white sons (14) were much fewer than the eosin (37). The culture which produced the bar-deficient exception (table 1) contained no such lethal, as is shown by the equality of the sexes (130♀ : 135♂) and by the equality of all contrary classes. The appearance of an inequality of sexes and of contrary classes indicates that whatever cause had removed or transformed the bar of the white bar chromosome was sufficiently damaging so that every male which received the deficient chromosome was unable to come to maturity. The deficiency was then not simply for bar but was also a deficiency for one or more genes whose normal action is essential to the life of the fly. The amount of linkage shown between the lethal effect and white is that expected on the view that the lethal change occurred at or near the bar locus. The white sons which did live were those which by crossing over in the mother $\left(\frac{w^e}{w}\right)$ had received from the eosin-bearing chromosome a normal piece to replace the damaged piece containing the region formerly occupied by bar and the vital allelomorphs.

STOCK OF DEFICIENCY

The possession of one deficient X $\left(\frac{\quad}{\quad}\right)$ does not kill the female, which is saved by the action of the dominant vital allelomorphs carried by the other X. Half of the daughters of such a female receive this deficient X and repeat the genetic results of their mother. A stock of deficiency was kept by selecting in each generation the daughters containing the deficient X, and, as is the practice with all lethals, this selection was rendered certain by linkage.

THE INCLUSION OF FORKED IN THE DEFICIENT REGION

It now seemed probable that the X chromosome of that particular sperm which gave rise to the exceptional daughter must have lost[4] a fragment containing the bar gene and also one or more genes necessary to the life of the animal. If this were the true explanation the chromosome should be deficient for all the genes within a definite distance of bar. It was anticipated that the section might be long enough to include more known loci than bar and the vital allelomorphs, and accordingly systematic tests were carried out with the mutations whose genes lie in the neighborhood of bar. The male, having but one X chromosome, is normally haploid with respect to all sex-linked genes; those females which have one normal X and one X deficient in the locus for a particular gene should behave with respect to that gene as if haploid and not as if diploid. That is, any recessive character whose locus lies opposite the deficient region should show itself in such a heterozygous female in spite of the fact that the character is normally a strict recessive, for in the deficient region there should be no active allelomorph to dominate.

Accordingly the next step was to test females carrying the deficient X, by forked, by rudimentary, and by fused, these being the recessive mutants with loci closest to bar (see map p. 457). The expected result was obtained with forked. Females heterozygous for deficiency, when outcrossed to forked males, gave half of their daughters forked, though these daughters were only heterozygous for forked! These forked daughters were derived from that half of the eggs which retained the deficient X, as was proved by the lethal result, as well as by the linkage relations shown by these forked females when they were bred (tables 4-8). This evidence shows that the mutation which removed the gene for bar and the vital allelomorphs, involved not simply these genes but also the normal genes of a section of the chromosome extensive enough to include the locus for forked.

THE MAXIMUM AND THE MINIMUM LENGTHS OF THE DEFICIENT REGION AS MEASURED BY THE HAPLOID TESTS WITH THE RECESSIVES

In the case of the other recessives (rudimentary and fused) the result obtained with forked did not occur (tables 4 and 5, appendix), which shows that the maximum length of the deficient region is the interval be-

[4] Since the results expected from complete inactivation are practically indistinguishable from those due to physical loss, consideration of the inactivation alternative will be deferred to a special section.

TABLE 3

The inclusion of forked in the deficient region as shown by tests of deficient-bearing females ($\frac{w}{\quad}\ \frac{-}{\quad}$) *by forked males.*

Culture	Daughters		Sons $\frac{w}{\quad}\ \frac{-}{\quad}$			
			$\frac{w}{\quad}$	$\frac{-}{\quad}$	$\frac{w}{\quad}$	$\frac{-}{\quad}$
No.	$f!$	+	Dies	+	w	Dies
668	49	55	—	24	28	—
669	79	72	—	41	25	—
691	129	107	—	76	46	—
692	128	114	—	66	47	—
Total	385	348	—	207	146	—

tween and exclusive of these two recessives, which are the nearest unaffected characters on either side. This maximum length is about 4.4 units. These same tests together with the data from the origin of deficiency show that the minimum length is the interval between and including forked and bar (about half a unit). The test with fused, which lies nearer the end of the chromosome than forked and bar (see map p. 457), brought out another significant point, namely, that the deficient X had not lost the entire end of the chromosome, but rather was deficient for a section near the end, leaving the genetic materials unchanged beyond this region. By crossing over between the deficient region and fused, females have been obtained in which the deficient X carries fused in the normal piece beyond the deficient region (table 6, appendix).

THE ABSENCE OF CROSSING OVER WITHIN THE DEFICIENT REGION

It seemed probable that the lethal effect of deficiency was not simply due to the deficiency for forked and bar, but rather that the deficiency had affected all the loci between forked and bar, including one or more whose normal action was essential to the life of the fly. In general it is to be anticipated that cases of deficiency should act as lethals, since it seems probable that any very extensive piece includes a locus vital to the animal. Very small deficiencies might conceivably fail to include such a vital locus, and it was thought that such smaller sections of the forked-bar deficiency might be obtained by crossing over in a female carrying forked—bar deficiency in one X and forked and bar in the other X. ($\frac{\quad}{f\ \ B'} \Rightarrow \frac{\quad\ B'}{f\ \ -}$) In an effort to test this possibility 1716 males were raised, but these did not show any crossovers (table 14, ap-

pendix). Either all crossover males died because of having a lethal fragment of the deficient region, or there were no crossovers to live or die. In testing this question an experiment (table 9, appendix) was devised so that the crossovers among the females could be detected, there being no question that the crossover females would live. There were raised 3138 such females and not one of them was a crossover! In this number of females 16 were expected to be crossovers. It is evident that crossing over in the deficient region is abnormally low, if indeed there is any crossing over whatever in this region. This disappearance of crossing over from the deficient region is practically proof that the entire region was involved, a section between and including forked and bar and certain vital genes.

THE EXTENT OF THE DEFICIENT REGION MEASURED BY LINKAGE

It was found that crossing over in other parts of the deficient X was of approximately normal frequency (table 14, appendix), and this fact made it possible to find out more nearly the maximum length of the deficient region. The elimination of genes and of crossing over from the deficient region results in a shortening of the genetic chromosome by an amount equal to the length of the deficient region. How much closer together have rudimentary and fused been brought by the occurrence of deficiency? Normally there is 4.4 percent of crossing over between rudimentary and fused. When deficiency was present there was a drop of 0.7 unit in this value (table 10, ♀♀, and table 14). This drop of 0.7 unit is in agreement with the previous data which showed that the minimum length of the deficient region is 0.5 unit. Evidently, however, the deficient region does not extend much beyond the forked—bar section.

THE DOMINANCE RELATIONS OF BAR AND THE NATURE OF THE BAR-DEFICIENT MUTATION

The mutative process, of which the first detected effect was (1) the loss of the dominant bar gene, involved also (2) the loss of the normal allelomorph of the recessive mutation forked, whose locus is about half a unit distant from that of bar, and (3) the loss of certain vital allelomorphs, which, from the linkage relations probably occupied the section between forked and bar; and (4) the disappearance of crossing over from this same region, due presumably to the loss of the physical framework of the chromosome. All of these four distinct but correlated effects can therefore be met and explained by the single hypothesis of the physi-

cal loss of a definite section of chromosome which has been measured by two genetic methods. It seems unreasonable that one of these effects should be due to a cause different from that of the others but initiated at the same time, as, for example, that the apparent loss of the bar gene (B') should have been in reality due to a remutation to its original wild-type allelomorph (b'), a process which will in nowise account for the other observed changes which had their origin in the same region at the same time.

It will be recalled that the eye shape of the normal heterozygous bar female ($\frac{B'}{b'}$) is an intermediate between the narrow bar of the homozygote and the round eye of the wild female. It was found (table 7, appendix) that the female carrying bar in one X and bar-deficiency in the other ($\frac{\quad}{B'}$) was in somatic appearance like the normal bar heterozygote and not like the homozygous bar female. The fact that the bar male with its one X chromosome carrying a single bar gene has an eye practically as narrow as that of the homozygous bar female, shows that one bar gene is sufficient to produce a fully narrow eye. That the bar gene of the $\frac{\quad}{B'}$ female does not make her eye fully narrow must be due to some opposing action tending to broaden the eye. Since the other X is deficient for the bar locus this broadening action must be due to genes outside of the bar locus. In the broad-eyed $\frac{\quad}{b'}$ female there are two sets of such broadening genes but only one narrowing gene (B'), while in the narrow-eyed homozygous bar female there are two narrowing genes. The narrowness of the eye of the bar male can be ascribed to its having the same ratio of narrowing action (1 B' gene: 1 set of broadening genes) as has the narrow-eyed homozygous female (2 B' genes: 2 sets of broadening genes). Likewise the broadness of the normal heterozygous bar female is not due to the action of the b' gene, as formerly supposed, but is due to the half ratio of narrowing to broadening genes (1 B' gene: 2 sets of broadening genes).

The suggestion that the broad-eyed $\frac{\quad}{B'}$ females owed their broadness to being in reality $\frac{-b'}{B'}$ females, due to the occurrence of a crossover ($\frac{\quad}{b'} \rightarrow \frac{-\,|\,b'}{|\,-}$) somewhere in the ancestry, is met by the evidence that in the ancestry opportunity to become such a crossover had been open to but a single female, and that she should be

such a crossover is incredible in view of the fact that in an experiment to test the amount of crossing over in the deficient region not one female in a total of over three thousand had proved to be a crossover.

The mutation responsible for deficiency may not have been, as so far assumed, the physical loss of a section of the chromosome; it may have been some kind of "inactivation," such as an internal rearrangement with change of properties, the loss of essential materials, or the addition of inhibiting agents. If inactivation is the explanation of deficiency then it must be complete inactivation; for in every case in which characteristics have disappeared, they have disappeared entirely. Thus, for example, the dominant bar gene retains no trace of its narrowing effect: the eye of a heterozygous female ($\frac{\quad}{b'}$) is wholly round, and the eye of the $\frac{\quad}{B'}$ female is no narrower than that of the regular heterozygote. In favor of the inactivation view may be cited the striking analogy with the Y chromosome: the Y, while cytologically of the same nature as other chromosomes, is genetically of little significance, as is proved by the fact that the effect of sex-linked genes in the male is in no case altered by anything in the Y, by the fact that the supernumerary Y's obtained through non-disjunction are without effect upon the visible characters, and by the lethal effect,—a fly having one or two Y's but no X being unable to live. Furthermore, there is no crossing over between Y and X even when the Y is in a female (XXY) in which there is certainly synapsis between Y and X and in which the occurrence of crossing over between the other chromosomes shows that the failure of crossing over in the case of the Y is due to a peculiarity of the Y itself. Since the Y offers a case of a chromosome inactive with respect to both the genetic materials and the framework, it becomes possible to suppose that the case of deficiency is an example of the same process that has produced the inactivation of the Y. That a piece of the Y chromosome has actually been substituted for the corresponding piece of the X seems impossible because such a substitution would involve three very improbable occurrences, viz., (1) crossing over in the male, (2) crossing over of the Y chromosome, and (3) a double crossover embracing a section only about a half unit long, while the shortest section in which a double crossover is known to have occurred is 13.5 units long.

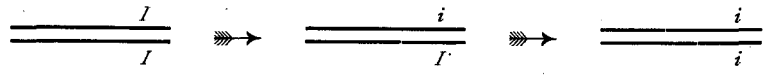
On the other hand, the "loss" view offers a more comprehensible solution for the fact that character genes different in nature but adjacent in position were affected by the one mutative change, and also for the fact that this one mutation affected, at the same time, the crossing over,

which seems to depend upon the framework of the chromosome rather than upon the character genes. As evidence that pieces may be lost bodily from chromosomes and that fragments may join together, there may be offered two distinct cases of "duplication" (unpublished), a phenomenon, the explanation of which seems to be that a section taken from the mid-region of one X has become attached to the end of the other X, its mate. The mid-region of the latter chromosome is represented twice, once in the normal location and again at the end. It seems probable that the first X, from which the duplicating fragment was taken bodily, would show the characteristics of deficiency, though in the two cases of duplication there was no evidence as to the fate of these terminal sections.

It seemed that a cytological examination might definitely settle this question, for on the loss view of deficiency the chromosome should be visibly shorter by an amount corresponding to the section lost. If the lost section were long enough, a difference in length between the two X's of a female having one deficient X should be observable. As stated in the beginning, the stock of deficiency was lost before the examination could be made. Several new cases which are possibly deficiencies in various parts of the X and even in other chromosomes have arisen, and it is hoped that combined cytological and genetic studies of these cases will make the subject clearer.

DEFICIENCY AND THE "PRESENCE AND ABSENCE" HYPOTHESIS

By following up the clue that a certain observed change might be due to the loss of a section of chromosome, we have been able to demonstrate a number of new and unusual facts which were predictable on that basis, and have thereby made it highly probable that the correct explanation has been found. This case of deficiency therefore constitutes the first valid evidence upon the question of "presence and absence." And it is significant to notice that the occurrence of the deficiency of a considerable section of genes has not brought to light any visible mutative changes in the way of dominants, contrary to what might well be expected on the presence and absence hypothesis. This is the more significant when it is recalled that the deficient region included the locus for bar,—a known dominant mutation. According to the presence and absence hypothesis the original appearance of the dominant bar character was due to the loss from the chromosome of an inhibitor, thereby allowing the normal narrowing effect of the remaining complex to assert itself.



Round Half narrow Narrow

Now, it should make no difference whether this inhibitor were lost by a special loss involving only the inhibitor or whether it were lost because of being situated in a particular section which itself became lost. In other words, the chromosome which is deficient for the region carrying the inhibitor should allow the occurrence of the same narrowing effect that is allowed by the simple loss of the inhibitor. In point of fact, the deficiency of the region in which the inhibitor must be hypothecated does not produce an effect like that of the mutation responsible for bar. For, the female carrying one deficient X and one normal X shows no narrowing of the eye shape, and likewise the female carrying one deficient X and one bar X is no narrower in eye shape than a normal heterozygous bar. Thus, in the only case which has a direct bearing on the presence and absence hypothesis, it is seen that the expedient of the loss of inhibitors to explain the origin of a dominant mutation is of no avail.

If, however, the appearance of the bar character were due to the creation of a new presence, then of course the loss of this presence by deficiency should restore the original condition; but that advocates of "presence and absence" have little liking for this type of explanation of the origin of a dominant is evident from the lengths they go in some recent expositions to avoid the vexed question of the origin of presences.

DEFICIENCY AND SEX DIFFERENTIATION

With non-disjunction the proof was complete that two X chromosomes determine a female and one a male. However, it has been suspected that the determiner of sex is not the "X-as-a-whole," but that in some definite part or parts of the X there are specific sex-differentiators. The case of deficiency favors this view; for, an XX individual having one deficient X is a *female,* normal in appearance and function. Two intact X's are not necessary for the production of a female; that is, sex-production is a function of some particular part of the X rather than of the X as a whole. Sex-differentiation was not affected by the occurrence of deficiency because the differentiators are in some region of the X other than the section from forked to bar.

THE REALITY OF THE CHROMOSOME MAPS

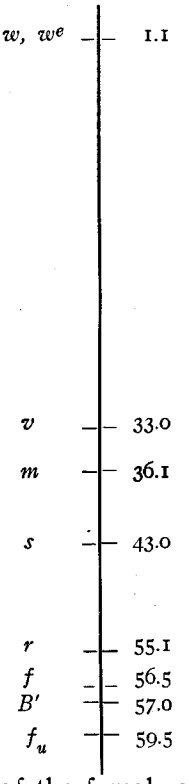
From the evidence of non-disjunction we know that the genes for the sex-linked characters are parts of the X chromosome. By means of

linkage studies we have been able to construct maps of the location of these sex-linked genes in a linear order which we believe to correspond to the linear structure of the chromosome. It has been objected that these maps may be only expressions of some "force" and that they do not correspond to an actual localization of genes along the chromosome. Deficiency furnishes the first direct evidence that the maps do correspond to a real localization of genes along the chromosome. That a single disturbing cause—the deficiency mutation—should exert a selective effect upon a certain few genes—bar, forked, vital allelomorphs—while leaving numerous other genes unaffected must be due to the possession by the few of a similarity either of properties or of location unshared by other genes. Nothing of the known properties of the dominant bar eye shape, the recessive forked bristle modification, and of the vital allelomorphs, are unique or suggest marked similarity to each other or dissimilarity between them and other sex-linked genes. *With regard to the location, however, there is independent and conclusive evidence from the linkage that these genes constitute a definitely localized and measurable section of the X chromosome.* The deficiency mutation was discovered because of its effect upon a single gene, viz., bar. It was then found to have affected at the same time one or more vital allelomorphs. When these vital allelomorphs are mapped according to the linkage shown, they are seen to occupy the region adjacent to bar. The argument from the location of forked within the deficient region is still stronger, for this location was detected and proved as the result of a deliberate search among those genes which had *previously been mapped closest to bar!* Nor does the evidence stop here, for not only did the deficiency mutation affect a section of adjacent genes but it also removed the crossing over from a definite section of chromosome. Now when the section from which the crossing over has been removed is compared upon the map with the section in which the genes are affected the two are seen to be identical. For a definite section of genes an identity has been established between the map and an actual distribution of genes.

APPENDIX

The sex-linked mutants referred to in this paper are: white eye color (w); eosin eye color (w^e), allelomorphic to white, and giving in females carrying white in one X and eosin in the other $\left(\frac{w}{w^e}\right)$, an intermediate eye color, "white-eosin compound"; vermilion eye color (v); miniature wings (m); sable body color (s); rudimentary wings (r);

forked bristles (f); bar eye shape (B'); fused venation (f_u); and deficiency for the region between and including forked and bar (—). Flies which show no mutant characters are said to be wild-type (+). The symbols included in parentheses do double duty, both to represent the genes for the mutants, and to tell the somatic appearance of flies (table headings, etc.); the small letters represent recessive mutants, and the primed capitals dominant mutants. The localization of these genes along the X chromosome as calculated from the linkage relations is given by the accompanying map[5] in which one unit of distance is one percent of total crossing over.



The two X chromosomes of the female are represented by two parallel lines with symbols showing the relative positions of the mutant genes involved ($\frac{w}{v}$), or more often by a single line, in which case the space above the line with its symbols represents one X and the space below, the other X ($\frac{w}{v}$). The chromosome which has the mutant gene farthest to the left of those involved in the cross is arbitrarily

[5] From MORGAN and BRIDGES 1916, Carnegie Institution of Washington publication 237.

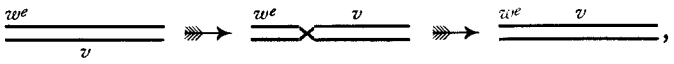
represented in the top space. The symbol $\frac{we}{v}$ is a contraction of  , and denotes that, by crossing over in a female which carries eosin in one X and vermilion in the other, the two eggs, eosin vermilion and wild-type, are produced. The zygote arising from the egg represented by the upper space is always written in the column to the left in the double column beneath the crossover symbol; likewise the column to the right corresponds to the egg of the lower space. By following this convention, we may often omit from the tables the individual headings of the two included columns (e.g., see table 11). In place of the crossover symbol, it is often advantageous to use the "crossover formula" as in table 13. Thus, in the first case in table 13, $\left(\frac{we \quad f}{vm \quad B'}\right)$, the "0" represents the sum of the flies that came from the two non-crossover gametes, eosin forked and vermilion miniature bar; the "1" represents the single crossovers between eosin and vermilion, that is, in the "first crossing over region"; the "1, 3 double crossovers" represent the flies resulting from the gametes $\frac{we \mid vm \mid f}{\quad \mid \quad \mid B'}$; etc.

TABLE 4

The non-inclusion of rudimentary in the deficient region as shown by the tests of deficient-bearing females by rudimentary males.

Forked females $\left(\frac{we \quad —}{\quad f}\right)$ *from culture 857, table 9A.*

No.	Daughters		Sons*			
			$\frac{we \quad —}{\quad f}$		$\frac{we \mid f}{\quad \mid —}$	
	+	r	Dies	f	wef	Dies
994	42	0	—	22	10	—
997	116	0	—	29	23	—
998	79	0	—	26	22	—
999	147	0	—	35	32	—
Total	384	0	—	112	87	—

* When females are heterozygous for deficiency and for forked, crossing over in the deficient region (if it occurs) should give the two contrary classes, non-forked sons and forked sons $\left(\frac{\quad —}{f} \mid \frac{}{—}\right)$. These sons should live only if they escaped retaining a lethal fragment of the deficient origin. The observed total lack

of any non-forked sons among the offspring is then additional evidence that no crossovers occur, or that these crossovers die. However, the forked crossovers would not be distinguished from the forked non-crossovers, and therefore this sort of evidence is equivalent only to a half amount as compared with the data of tables 6 and 8. Accordingly, in table 14, the data from males on crossing over in the deficient region includes all the males of tables 6 and 8, but only half the males of tables 4, 5, 7, 9, and 9 A.

TABLE 5

The non-inclusion of fused in the deficient region as shown by the tests of deficient-bearing females by fused males.

Forked females ($\frac{w^e \quad —}{f}$) *from culture 857, table 9A.*

No.	Daughters		Sons			
			$\frac{w^e \quad —}{f}$		$\frac{w^e \mid f}{—}$	
	+	f_u	Dies	f	wef	Dies
988	118	0	—	37	13	—
989	71	0	—	27	16	—
991	206	0	—	50	44	—
993	175	0	—	40	27	—
Total	570	0	—	154	100	—

TABLE 6

The insertion of fused into the intact region beyond the deficient region.

No.	♀♀	♂♂*			
		$\frac{\quad f_u}{f \quad B'}$		$\frac{\quad \mid f_u}{f \quad B'}$	
		Dies	fB'	Dies	$fB'f_u$
1755	134	—	58	—	1
2176	30	—	11	—	1
Total	164	—	69	—	2

* These males are not included in the summary of table 14 as showing the amount of crossing over between deficiency and fused, for there might be other like cultures (table 8) which failed of detection because no crossover occurred. A crossover value calculated from such incomplete data could only be regarded as a maximum value.

TABLE 7

Tests of the relation between deficiency and bar by outcrossing deficient-bearing females (from culture 668, table 3) to bar males.

No.	♀♀	♂♂			
		$\frac{w^e \quad —}{\quad f}$		$\frac{w^e \mid f}{\quad —}$	
	B'	Dies	f	$w^e f$	Dies
749	60	—	17	11	—
751	24	—	8	3	—
754	52	—	19	15	—
Total	136	—	44	29	—

TABLE 8

The non-occurrence of crossover sons of deficient-bearing mothers heterozygous for forked and bar ($\frac{\quad}{f \quad B'}$).

No.	Daughters	Sons			
		$\frac{\quad \quad}{f \quad B'}$		$\frac{\quad \mid B'}{f \mid \quad}$	
		Dies	fB'	B'	f
1200	51	—	33	—	—
1313	81	—	35	—	—
1752	97	—	25	—	—
1753	79	—	43	—	—
1754	114	—	64	—	—
1756	54	—	38	—	—
1757	41	—	26	—	—
1981	130	—	57	—	—
1981r	39	—	21	—	—
2177	41	—	31	—	—
Total	727	—	373	—	—

TABLE 8A

Mothers heterozygous for sable also ($\frac{s\ \ f\ \ B'}{\quad\quad}$).

		$\frac{s\ \ f\ \ B'}{\quad\quad}$		$\frac{s\ \ \ \ }{\ \ f\ \ B'}$		$\frac{s\ \ f\ \ \ }{\ \ \ \ B'}$	
		sfB'	Dies	Dies	*fB'*	*sf*	*B'*
1145	124	50	—	—	12	—	—
1758	78	17	—	—	3	—	—
1759	38	17	—	—	2	—	—
1764	107	35	—	—	10	—	—
1765	97	31	—	—	4	—	—
1766	36	12	—	—	4	—	—
1767	113	49	—	—	11	—	—
Total	593	211	—	—	46	—	—

TABLE 9

The non-occurrence of crossover daughters when deficient-bearing mothers heterozygous for bar ($\frac{\quad\quad}{B'}$) *were backcrossed to forked males.*

No.	Daughters				Sons			
	$\frac{\quad\quad}{B'}$		$\frac{\ \ \ \ }{\ \ \ \ }\ B'$		$\frac{\quad\quad}{B'}$		$\frac{\ \ \ \ }{\ \ \ \ }\ B'$	
	f	*B'*	*fB'*	+	Dies	*B'*	*B'*	+
2222	77	90	—	—	—	76	—	—
2223	69	66	—	—	—	49	—	—
2224	60	64	—	—	—	69	—	—
2234*	75	64	—	—	—	69	—	—
2235	77	81	—	—	—	61	—	—
2236	51	50	—	—	—	34	—	—
2238	51	71	—	—	—	71	—	—
2239	68	53	—	—	—	65	—	—
2240	82	61	—	—	—	49	—	—
2241	54	59	—	—	—	46	—	—
2246	77	70	—	—	—	58	—	—
2260	85	82	—	—	—	113	—	—
2261	42	32	—	—	—	11	—	—
2264	86	73	—	—	—	81	—	—
2265	62	90	—	—	—	76	—	—
2286	48	49	—	—	—	56	—	—
2290	93	127	—	—	—	111	—	—
2316	79	106	—	—	—	86	—	—
2320	37	70	—	—	—	77	—	—
Total	1273	1358	—	—	—	1258	—	—

TABLE 9 A

Mothers heterozygous for eosin also ($\frac{w^e \quad —}{\quad B'}$).

					$\frac{w^e \quad —}{\quad B'}$		$\frac{w^e \quad B'}{\quad —}$		$\frac{w^e \quad —}{\quad B'}$	
					Dies	B'	w^e	Dies	w^eB'	+
857	60	83	—	—	—	48	24	—	—	—
858	108	121	—	—	—	48	47	—	—	—
859*	60	75	—	—	—	40	29	—	—	—
Total	228	279	—	—	—	136	100	—	—	—

* In each of the cultures 2234 and 859 a sterile forked male (XO) appeared, due to primary non-disjunction.

TABLE 10

The crossing over in deficient-bearing females heterozygous for rudimentary and fused ($\frac{r \quad f_u}{\quad —}$).

No.	Daughters				Sons*					
	$\frac{r \quad f_u}{\quad —}$		$\frac{r \quad —}{\quad f_u}$		$\frac{r \quad f_u}{\quad —}$		$\frac{r \quad —}{\quad f_u}$		$\frac{r \quad —}{\quad f_u}$	
	rf_u		r	f_u	rf_u	Dies	Dies	f_u	r	Dies
1437	17	80	0	2	20	—	—	0	1	—
1438	43	89	1	1	41	—	—	1	2	—
1599	27	51	3	1	40	—	—	2	0	—
1600	45	66	2	3	56	—	—	2	0	—
1601	38	59	1	1	37	—	—	1	0	—
1619	21	37	1	3	12	—	—	0	0	—
1696	18	37	0	2	16	—	—	0	0	—
1697	15	21	2	0	8	—	—	1	0	—
1700	21	26	1	3	10	—	—	0	2	—
Total	245	466	11	16						
1775		80			16	—	—	1	1	—
1778		31			10	—	—	0	0	—
1779		67			16	—	—	0	1	—
1905		31			15	—	—	0	0	—
1906		31			19	—	—	0	1	—
2106		100			32	—	—	4	1	—
2107		67			14	—	—	0	1	—
2175		56			31	—	—	1	1	—
Total		463			393	—	—	13	11	—

* The males of table 10 are not included in the summary of table 14, because of the difficulty of calculating the true amount of crossing over when a lethal (deficiency) and a poorly viable mutant (rudimentary) are both present. Ordinarily the poor viability of a mutant has little effect upon the apparent amount of crossing over, because, in each pair of contrary classes, the relative smallness of the class in which the non-viable mutant occurs is counterbalanced by the relative largeness of the contrary class in which its viable normal allelomorph occurs. This is the case with the females

of table 10. But when a lethal also is present (as in the case of the males of table 10), the lethal kills one of each pair of contrary classes and hence certain classes are relatively too low (as, in table 10, the non-crossover class rudimentary fused and the "2" crossover class rudimentary), while certain other classes are relatively too high (as the "1" crossover class fused). That the males of table 10 are in agreement with the females is evident after a correction has been made for the disturbance due to the unbalanced non-viability. The females of table 10 gave the interval between rudimentary and fused as 3.7 units, instead of the normal 4.4, a decrease of .7 unit due to the deficient region. The percentage expectation for the males on this basis (the distance from rudimentary to the deficient region = 1.4, the length of the deficient region = .7 unit, and deficiency to fused = 2.3) is "0" = 96.3, "1" = 1.4, and "2" = 2.3. These percentages become "0" = 94.24, "1" = 3.11, and "2" = 2.65 if 44 percent of the rudimentary and 88 percent of the fused zygotes hatch. The observed percentages are "0" = 94.24, "1" = 3.12, and "2" = 2.64. The assumed percentages of viability of rudimentary and of fused are in agreement with the results of other experiments in which these mutants are involved.

TABLE 11

The linkage of white and deficiency.

No.	Daughters	$\frac{w}{-}$		$\frac{w}{-\mid -}$	
632	166	—	34	14	—
642	123	—	40	35	—
645	264	—	82	53	—
647	216	—	59	35	—
731	58	—	32	12	—
Total	827	—	247	149	—

TABLE 12

The linkage of sable and deficiency.

$\frac{s}{-}$ *females backcrossed to sable forked males.*

No.	Daughters				Sons			
	$\frac{s}{-}$		$\frac{s}{-\mid -}$		$\frac{s}{-}$		$\frac{s}{-\mid -}$	
	s	*f*	*sf*	+	*s*	Dies	Dies	+
1024	34	39	5	8	31	—	—	5
1025	27	26	5	7	21	—	—	6
1026	34	39	6	9	33	—	—	6
1201*	41	52	8	11	49	—	—	3
1242	73	73	8	14	72	—	—	7
1244	29	20	5	3	27	—	—	6
Total	238	249	37	52				
867	111				58	—	—	11
900	81				35	—	—	5
975	88				44	—	—	10
1120	83				41	—	—	11
Total	363				411	—	—	70

* In culture 1201 a sterile sable forked male (XO) appeared, due to primary non-disjunction.

TABLE 13

Incidental linkage data not involving deficiency.

Genes	No.	Classes with respect to crossing over						
$\frac{w^e \quad f}{vm \quad B'}$	862	0	1	2	3	1, 3	2, 3	1, 2, 3
		45	30	2	23	8	1	1
$\frac{w^e \quad f}{B'}$	861	0				1		
		42				22		
$\frac{v\,m \quad B'}{f}$	863	0			1		2	
		68			2		31	
$\frac{s}{f}$	864	0				1		
		102				27		
	865	123				22		
	866	92				18		
	868	80				9		
	869	137				15		
	870	139				25		
	871	75				15		
	872	131				19		
	873	77				9		
	885	44				7		
	1235	152				27		
	1243	267				39		
	Total	1419				232		
$\frac{r}{B'}$	2263	0				1		
		153				3		
$\frac{r \quad f_u}{B'}$	2242	0		1		2		
		111		3		8		
	2243	66		1		2		
	2245	24		1		2		
	2262	15		1		0		
	2267	192		0		7		
	2287	114		3		5		
	2289	131		2		5		
	2291	132		1		3		
	2317	112		3		1		
	2318	157		4		5		
	2319	85		2		1		
	2352	94		1		3		
	Total	1233		22		42		
$\frac{r}{f_u}$	1174	0				1		
		49				0		
	1175	20				0		
	1207	110				3		
	1208	91				5		
	1209	101				3		
	1281	106				3		
	1282	47				1		
	1308	113				4		
	Total	637				19		

TABLE 13 (Continued)

Genes	No.	Classes with respect to crossing over	
		0	1
$\frac{f}{B'}$	860	379	1
	1021	163	3
	1022	163	1
	Total	705	5
$\frac{B'}{f_u}$		0	1
	2244	100	4

TABLE 14

A summary of the linkage data of this paper.

Genes	Total	Crossovers	Percent
w v	110	39	35.4
w m	110	41	37.3
w —	1562	625	40.0
w f	174	78	44.8
w B'	174	78	44.8
v m	211	6	2.8
v f	211	66	31.3
v B'	211	66	31.3
m f	211	64	30.3
m B'	211	64	30.3
s —	1314	205	15.6
s f	1651	232	14.0
r B'	1453	25	1.7
r f_u	1953	83	4.3
$\left(\frac{r \quad f_u}{\quad}\right)$	738	27	3.7
f B'	980	5	0.5
$\left(\frac{f \quad B'}{\quad}\right)$	♂♂ 1716	0	0.0
	♀♀ 3138	0	0.0
B' f_u	1401	46	3.3