VERMILION-DEFICIENCY.

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CONTENTS.

SUMMARY.

In May, 1916, a culture *of Drosophila melanogaster* showed that a new sex-linked lethal had arisen. The linkage relations indicated that the position of the lethal was in the neighborhood of the sex-linked recessive "vermilion," whose locus in the X chromosome is at 33.0. When females heterozygous for the lethal were outcrossed to vermilion males, all the daughters that received the lethal-bearing chromosome showed vermilion eye-color, though, from the pedigree, vermilion was known to be absent from the ancestry of the mother. The lethal action and the unexpected appearance of vermilion both suggested that this was another instance of the phenomenon called "deficiency;"[1] that is, the loss or "inactivation" of the genes of a section of the X chromosome. The lethal action would then be due to the deficient region including one or more genes necessary for the life of the individual. The appearance of vermilion in females carrying only one vermilion gene would be explainable on the ground that the deficient-bearing

[1] Bridges, C. B., *Genetics*, 1917, ii, 445.

females are virtually haploid for the region including the vermilion locus. Linkage tests showed that the amount of crossing over in the neighborhood of the deficiency was cut down by about five units. Part of this may be attributed to the actual length of the "deficient" region, within which it is probable that no crossing over occurs, and part (probably most) to an alteration in the synaptic relations in the regions immediately adjacent. In more remote regions there was no disturbance or perhaps a slight rise in the frequency of crossing over. Both the local fall and the possible rise in more distant regions would seem to argue that a "pucker" at synapsis had been caused by an actual shortening of the deficient chromosome. That the deficient region extends to the left of the locus of vermilion was indicated by a test in which it was observed that the presence of an extra piece of chromosome including the loci for vermilion and sable ("vermilion-sable duplication") did not neutralize the lethal action of the deficiency. Haploid tests with the other recessive mutations in the neighborhood of vermilion showed that the deficiency was not extensive enough to include their loci. Cytological preparations were made but were unsatisfactory. The stock was finally lost, apparently as the result of injurious action upon viability, fertility, and productivity by the deficiency.

References in this paper are made to the following recessive sex-linked mutations: "facet" eye (*fa*, locus 2.4), "cut" wing-shape (*ct*, 20.0), "ocellar-bristles" lacking (*ob*, 32.—), "vermilion" eye-color (*v*, 33.0), "tiny-bristles" (*tb*, 36.0), "miniature" wing-size (*m*, 36.1), "sable" body-color (*s*, 43.0), "garnet" eye-color (*g*, 44.4), "forked" bristles (*f*, 56.5). The tentative symbol for "vermilion-deficiency" is $-(33.\pm)$, for "vermilion-sable duplication" *V-S* (0.0), and for "sable-duplication" **S** (0.0). The symbol + is read "wild-type." Sable-duplication is equivalent to a wild-type allelomorph of sable situated at the extreme left end of the X; males of the constitution $\frac{\mathbf{S}}{\quad}\ \frac{s}{\quad}$ are accordingly wild-type in appearance. Likewise *V-S* duplication "covers" the recessive genes vermilion and sable.

Origin and Lethal Action of Vermilion-Deficiency.

The recessive sex-linked mutation tiny-bristles had arisen in the early work on "sable-duplication" (unpublished data). It was not then convenient to give time to working out the locus of tiny-bristles

very accurately, so that a stock of tiny-bristles was carried along in the easiest way by breeding tiny-bristle males to their heterozygous sisters. Tiny-bristle females were found to be so infertile that they could not be depended upon. One of the parents of such a stock culture was a female carrying in one X chromosome tiny-bristles, garnet, and forked, and, in the other X, sable-duplication, sable, and garnet $\left(\frac{\mathbf{S}\ \ s\ g}{tb\ \ g\ \ f}\right)$. The other parent was a male carrying sable-

TABLE I.

Offspring of Garnet Female, Constitution $\left(\frac{\mathbf{S}\ \ s\ g}{tb\ \ g\ \ f}\right)$, *and Garnet Forked Male, Constitution* ($\underline{\mathbf{S}\ \ g\ \ f}$).

Feb. 14, 1916.

Culture No.	Females.		Males.						
	g	*gf*	*g*	*tb gf*	*sg*	*tb sg*	*gf*	*tb g*	*sgf*
3,302	20	14	7	10	2	1	5	1	1

TABLE II.

Offspring of Garnet Female, Constitution $\left(\frac{\mathbf{S}\ -\ g\ \ f}{\mathbf{S}\ \ s\ g}\right)$, *and of Tiny-Bristle Garnet Forked Male* ($\underline{\mathbf{S}\ \ tb\ g\ \ f}$).

Mar. 9, 1916.

Culture No.	Females.		Males.	
	g	*gf*	*g*	*gf*
3,680	51	57	44	11

duplication, garnet, and forked ($\underline{\mathbf{S}\ \ g\ \ f}$). The offspring of this pair were of the expected kinds and frequencies (Table I). No lethal was present.

For the next generation a garnet female was crossed to a tiny-bristle garnet forked brother. The offspring showed that this male had received sable-duplication from the mother as the result of crossing over, and was of the constitution $\underline{\mathbf{S}\ \ tb\ g\ \ f}$. The mother was expected to carry sable-duplication, sable, and garnet in one X and

sable-duplication, garnet, and forked in the other X $\left(\frac{\mathbf{S}\ \ s\ g}{\mathbf{S}\ \ g\ f}\right)$.

The progeny (Table II) agreed with the expectation except that in addition to these genes of the mother a new lethal had appeared which killed off half the males. The lethal gene had appeared by mutation in the $\underline{\mathbf{S}\ g\ f}$ X derived from the father, and its locus was 20 or more units from forked on the basis of the eleven crossovers in a total of 55 sons. This distance is great enough so that it was thought probable that the locus of the lethal was to the left of forked rather than further toward the distal end of the chromosome. No great importance was attached to this occurrence of a lethal, since they occur frequently; no less than five were detected in the early work on sable-duplication.

In the next generation a start was made to determine the locus of tiny-bristles, the slight information already in hand having indicated a position some 6 or 8 units to the left of sable; that is, in the neighborhood of miniature. It was thought more advisable to use vermilion before miniature, since there was some chance that the small size of the wings of the tiny-bristle mutation would interfere with the classification of the miniature wing character. Accordingly, some of the garnet daughters of Culture 3,680 were outcrossed to vermilion forked males. Eight of these daughters gave progeny in accordance with the constitution $\left(\frac{\mathbf{S}\ \ tb\ \ g\ \ f}{\mathbf{S}\ \ \ \ s\ g}\right)$ (Table III) which furnished data with respect to the amount of crossing over between tiny-bristle and forked.

However, two daughters (Table IV) gave results different in two respects: the lethal found present in the parental culture was here evidenced in the fact that there were only half as many males as females (86♂ : 181♀), and in the fact that the locus of this lethal corresponded to the position calculated for the lethal present in the previous generation, since its location was 19 units to the left of forked and about 2 units to the left of tiny-bristles. The mothers of the two cultures had come from eggs representing normal crossing over between the lethal and sable in Female 3,680. All the above results are normal; however, a totally unexpected feature appeared in the

fact that *half the females were vermilion although there had been no vermilion in the maternal ancestry.* The amount of crossing over between this vermilion and forked (19.9 per cent) was slightly less than the amount expected from ordinary vermilion and forked. But there was no crossing over observed between the vermilion and the lethal; that is, there were no vermilion sons. This set of facts immediately suggested that here was another case of "deficiency"[1]—

TABLE III.

Offspring of Eight Females, Constitution $\left(\frac{S \quad tb \quad g \quad f}{S \quad\quad s\ g}\right)$ *from Culture 3,680, and Vermilion Forked Males.*

Mar. 26, 1916.

	Females.	Males.				
		tb g f	*g*	*tb g*	*g f*	*v f*
Total.....	1,021	192	380	75	76	1 (XO)

TABLE IV.

Offspring of Two Garnet Daughters, Constitution $\left(\frac{S \quad - \quad\quad s\ g}{S \quad tb \quad g \quad f}\right)$ *from Culture 3,680, and Vermilion Forked Males.*

Mar. 26, 1916.

Culture No.	Females.				Males.		
	v/	*f*	*v/f*	+	*tb g f*	*g*	*tb g*
3,987	39	36	7	14	44	1	10
3,989	34	36	7	8	26	1	4
Total.....	73	72	14	22	70	2	14

that the process which gave rise to the lethal was not simple mutation but a process characterized by the "*inactivation*" *or loss of the genes of a whole section of the X chromosome.* The garnet forked father of Culture 3,302, according to this view, had produced a sperm whose X was "deficient" for the vermilion locus and for a section of chromosome long enough so that one or more genes necessary for the life of the male individual were rendered inoperative. The deficiency of

these vital genes had given rise to the lethal effect, and the deficiency for the vermilion locus rendered a female carrying one such chromosome virtually haploid for vermilion. A female, one of whose X chromosomes carries vermilion-deficiency and the other the recessive vermilion gene, shows the vermilion character as does a male carrying the vermilion gene in its single X chromosome. From the facts in certain other cases of deficiency, especially Notch 8 (N 8),[2] it is probable that the condition is not as simple as just represented, but that the vermilion-deficiency has some positive action, in some ways comparable to, but more extreme than, the action of the vermilion gene.

Linkage Tests of the Extent of the Deficient Region and the Disturbance in Crossing Over in Adjacent Regions.

The work with the first deficiency[1] had shown that there was probably no crossing over within the deficient region, and that the chromosome map was shortened by an amount equal to the length of the deficient region. In certain other cases of deficiency, notably dachs-deficiency,[3] it has been found that besides this shortening equivalent to the length of the deficient region there is extensive alteration in the amounts of crossing over in neighboring regions of the chromosome. This disturbance seems to take the form of a marked decrease in the amount of crossing over in the immediate vicinity of the deficiency with perhaps slight increases in more remote regions. Such an effect would follow from disturbed synaptic relations and would seem to argue that deficiency involves a real contraction of the deficient chromosome, with resultant "puckers" at synapsis, rather than merely an inactivation.

The first step taken was to test whether there was marked reduction of crossing over in the region to the left of the supposed deficiency. The vermilion forked and lethal forked crossover values had already shown that there was probably not much disturbance in the long region to the right of vermilion. The recessive mutation facet whose locus is at about 2.6 was used to control the left end.

[2] Mohr, O. L., *Genetics*, 1919, iv, in press.

[3] Bridges, C. B., and Morgan, T. H., *Carnegie Institution of Washington Publication, 278*, 1919, pt. ii.

Three of the vermilion daughters of Culture 3,987 were outcrossed to facet vermilion forked males (Table V).

The males of Table V showed that there was probably a decrease of crossing over immediately to the right of the deficient region, since the vermilion-deficiency sable crossover value was 7.4, instead of 10.0, which is the normal vermilion-sable value.

TABLE V.

Offspring of Vermilion Females, Constitution $\left(\frac{S - sg}{v \quad f}\right)$, *Outcrossed to Facet Vermilion Forked Males.*

Apr. 16, 1916.

Culture No.	Females.	Males.				
		vf	*vsg*	*vg*	*v*	*vsgf*
4,236	257	98	11	—	20	—
4,237	222	76	9	2	11	—
4,258	229	81	3	5	8	1
Total.....	708	255	23	7	39	1

TABLE VI.

Offspring of Vermilion Females, Constitution $\left(\frac{fa \quad v \quad f}{- \quad sg}\right)$ *from Table V, Outcrossed to Cut Vermilion Forked Males.*

May 8, 1916.

Culture No.	Females.	Males.					
		favf	*vf*	*favsg*	*fav*	*vsg*	*v*
4,514	43	8	2	1	2	—	—
4,518	78	22	7	—	2	—	—
4,519	61	17	4	—	3	1	3
Total.....	182	47	13	1	7	1	3

Over a dozen vermilion-deficient females whose father was facet vermilion forked were outcrossed to cut vermilion forked males, though only three of these cultures produced offspring (Table VI). The resulting data were rather insufficient, but indicated that there was probably somewhat less than the normal amount of crossing over

in the facet to deficiency interval. There was again considerable reduction in the crossing over between the deficiency and sable which was here only 2.8 instead of 10.0 per cent.

The cut vermilion forked male had been used in the mating just described in order that in the next generation a closer analysis of the crossover reduction immediately to the left of the deficiency might be made.

TABLE VII.

Offspring of Vermilion Females, Constitution $\left(\frac{ct\ \ v\ \ \ f}{-\ \ s\ g}\right)$.

June 28, 1916.

Culture No.	Females.	Males.						
		ct v f	*v f*	*ct v s g*	*ct v g*	*ct v*	*v*	*ct v s g f*
4,676	89	36	6	2	—	4	—	—
4,583	81	13	2	—	1	1	—	—
4,684	117	24	4	1	1	3	—	—
4,686	41	14	—	1	—	3	—	1
4,687	22	6	2	2	—	—	—	—
4,703	53	12	2	—	—	6	—	—
4,704	44	14	2	3	—	2	—	—
5,105	67	31	4	5	—	6	—	—
5,164	44	9	—	2	2	2	—	—
5,166	49	23	3	1	—	3	—	—
6,783	88	22	6	2	—	4	—	1
6,784	55	18	2	1	—	3	—	—
6,902	83	27	1	1	4	3	—	—
6,903	165	61	14	5	1	14	—	—
6,727	108	33	6	8	—	6	1	—
6,945	96	34	5	2	1	3	1	—
Total.....	1,202	377	59	36	10	63	2	2

Considerable data have been collected showing the amount of crossing over in females carrying cut vermilion and forked in one X chromosome and deficiency sable garnet in the other (Table VII).

The most significant crossover values calculable from the results of Table VII are cut deficiency which is 11.1 instead of 13.0—a decrease of 1.9 units, and deficiency sable which is 6.9 instead of 10.0—a decrease of 3.1 units.

In Table VIII is given a summary of the more significant crossover values as determined from all the experiments involving deficiency, with normal values for comparison.

From these values it is apparent that there is a reduction in crossing over between cut and vermilion-deficiency of about 2 units, while there is a similar reduction between deficiency and sable of about 3 units, or a total shortening of the chromosome map of about 5 units. It is known that not all the reduction to the right of deficiency is due to the length of the deficient region, since crossing over occurred between deficiency and tiny-bristles. Tiny-bristles and vermilion are normally 3.0 units apart, while there were two crossovers in a

TABLE VIII.

Comparison of Normal Crossover Values With Those Obtaining for Females Heterozygous for Vermilion-Deficiency.

Loci.	Deficiency.	Normal.	Change.
ct —	11.1	13.0	−1.9
— *tb*	2.3	3.0	−0.7
— *s*	6.8	10.0	−3.2
— *f*	20.1	22.9	−2.8
s g	1.8	1.4	+0.4
g f	12.4	12.1	+0.3

total of 86 males, which showed that about 2.3 per cent of crossing over occurs between deficiency and tiny-bristles. This evidence does not allow us to determine the actual length of the deficient region nor what proportion of the deficiency is to the left of the vermilion locus and how much is to the right. The garnet forked value is normal or perhaps slightly high.

The normal values used for comparison in Table VIII are the mean values derived from several experiments[4] and are not, as would have been more desirable, from sister cultures. While the two sets of values are therefore not strictly comparable, the differences observed were so constant in direction that there can be little doubt of the reality of the changes.

[4] Morgan, T. H., and Bridges, C. B., *Carnegie Institution of Washington, Publication No. 237*, 1916; also unpublished data.

Relation between Vermilion-Deficiency and Vermilion-Duplication.

That the deficient region actually does extend to the left of the vermilion locus was indicated by use of vermilion-duplication. The stock of vermilion-duplication consists of flies homozygous for the vermilion gene and at the same time homozygous for the not-vermilion gene, since these flies carry at the left end (point of spindle-fiber attachment?) a transposed section of chromosome including the loci for vermilion and sable. These flies are not vermilion in appearance since the two wild-type allelomorphs dominate over the two recessive vermilion genes. But if such females are crossed to vermilion males all the daughters are vermilion and all the sons are wild-type—simulating the Abraxas type of "criss-cross" inheritance, or dominance of vermilion. These daughters are vermilion because two recessive vermilion genes are present and dominate over the single wild-type allelomorphs of the F_1 female. When vermilion females carrying vermilion in one X and deficiency in the other were crossed to males of the duplication stock, all the daughters were vermilion. In its effects upon the dominance of vermilion, vermilion-deficiency may be substituted for a vermilion gene without distinguishable difference. This fact also indicates a positive action of vermilion-deficiency. Had the deficiency been neutral in effect, then the flies carrying vermilion, not-vermilion, and vermilion-deficiency should have been wild-type like the normal diploid heterozygote.

The F_1 females were crossed to males from the vermilion-duplication stock (Table IX). The most significant point observed in F_2 was that the lethal effect of vermilion-deficiency was not annulled by the presence of the duplication. The probable explanation of this is that the two regions do not coincide; *i.e.*, although the duplicating fragment includes the loci from vermilion to the right as far as sable, nevertheless it does not extend so far to the left of vermilion as the deficiency does. This excess of the deficient region to the left of vermilion would have to be enough to include one or more loci vital to the animal.

It should be noticed that there was 35.5 per cent of crossing over between zero and vermilion-deficiency, which is about 3.5 units more than the average normal value.

TABLE IX.

Offspring of Vermilion Females, Constitution $\left(\frac{V-S\ v}{-\ \ s\ g}\right)$, *and Wild-Type Males, Constitution* $(V-S\ \ v\ \ )$.

Apr. 23, 1917.

Culture No.	Females.		Males.				
	+	*v*	+	*v*	*g*	*v s g*	*v g*
7,055	79	86	37	20	4	1	1
7,065	76	75	32	26	5	—	—
7,076	19	47	13	8	3	—	—
7,080	31	34	18	8	3	—	—
7,081	59	53	34	21	7	—	—
Total.....	264	295	134	83	22	1	1

Haploid and Cytological Tests of the Extent of the Deficient Region.

Another method of getting light on the extent of the deficient region is by testing whether the deficiency works for other mutations in the same manner as with vermilion. Tiny-bristles and miniature were known by the linkage tests to be outside the region so that the failure of both to give pseudodominance was not unexpected. The only other mutant whose locus was close enough to be worth while testing was "ocellar-bristles" whose locus is to the left of vermilion by one or more units—not accurately located because of the poorness of the character. This mutant also failed to give pseudodominance so that its locus lies outside the deficient region.

Considerable cytological work was undertaken, but none of the slides were good enough technically to be able to demonstrate the deficiency cytologically.

Dominant Action of Deficiencies.

The stock of vermilion-deficiency was unusually difficult to maintain, being on the verge of extinction several times before its final loss. The same fact was observed with respect to the orginal forked-bar-deficiency and all the notch stocks, which have been lost (ex-

cept Notch 8) after a longer or shorter period of struggling existence. This fact also would indicate a positive action on the part of deficiency such that the heterozygous females are of lower viability, fertility, and productivity, even when, as in the cases of forked- and of vermilion-deficiencies, no somatic character is observable.