THE LINKAGE OF EIGHT SEX-LINKED CHARACTERS IN *DROSOPHILA VIRILIS*

CHARLES W. METZ
Station for Experimental Evolution, Cold Spring Harbor, New York
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The present paper deals with eight sex-linked mutant characters that have arisen in my cultures of *Drosophila virilis* Sturtevant.[1] The eight characters are as follows:

Yellow, body color.
Magenta, eye color.
Glazed, eye surface (figure 5).
Forked, bristles on the thorax (figure 2).
Vesiculated, wings.
Rugose, eye surface (figure 4).
Frayed, bands on the abdomen (figures 3 and 6).
Hairy, eye surface (figure 7).

DESCRIPTION OF MUTANT CHARACTERS

The first four of these mutants have been described previously (METZ 1916) and may be passed over briefly. Yellow is distinguished by the yellowish color of the body and wings; magenta by its peculiar eye color; glazed (figure 5) by the varnished or glazed eye surface, and irregular ommatidia; and forked (figure 2) by the forked or stunted bristles on the head and thorax.

The remaining four characters are considered here for the first time, and in connection with their description a word may be said with respect to their origin and initial behavior.

Vesiculated is characterized by the presence of vesicles or blisters in the wings, somewhat like those in the "balloon" wing mutant of *D. ampelophila.* Occasionally the entire wing may be swollen into a single large vesicle filled with liquid, but usually the abnormality is limited to one or two small blisters near the center of the wing. This character was first observed (July, 1916) in several males from a mass culture of normal flies. These males when bred to normal unrelated females gave

[1] For a description of this species see STURTEVANT 1916.

Explanation of Plate I

The figures are all drawn to the same scale with the aid of a camera lucida, but the individuals were of various sizes due to different cultural conditions.

Figure 1. *Normal,* male. The wing veins and bristles are somewhat exaggerated in this figure.

Figure 2. *Forked,* female.

Figure 3. *Frayed,* male, dorsal view. The wings are cut off to show the abdomen.

Figure 4. *Rugose,* male.

Figure 5. *Glazed,* male.

Figure 6. *Frayed,* male, side view. Wings cut off to show abdomen.

Figure 7. *Hairy.*

Figure 8. *Normal.* Side view for comparison with figure 6. Wings cut off.

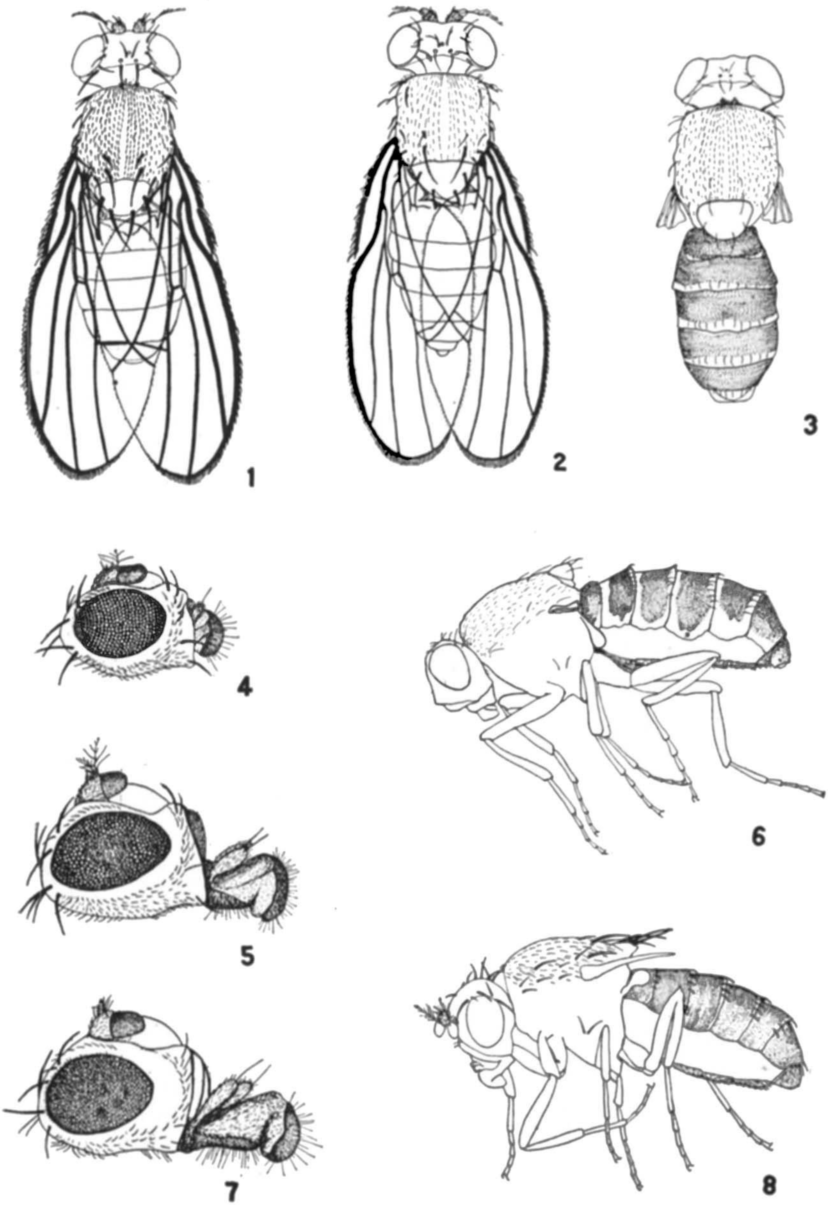


PLATE I

in F_1 normal females and males, and in F_2 224 normal females, 110 normal males and 97 vesiculated males. In F_3 vesiculated females were obtained and a pure stock made up which has subsequently bred true.

The modification called rugose (figure 4) somewhat resembles glazed, but is much less pronounced. The ommatidia of the eye are greatly disarranged but are not fused together in masses as they are in glazed, and there is no smeared or varnished appearance to the surface. Furthermore the eye is full-sized instead of narrow and is decidedly lighter than the normal or the glazed eye. Rugose first appeared in a single male individual (June, 1916) from a mating between a normal female and a confluent bald (non-sex-linked characters) male. This mutant, bred to normal females, gave in F_1 normal males and females, and in F_2 43 normal females, 18 normal males and 11 rugose males. Thus far the behavior was that of a typical sex-linked recessive, but the results in F_3 showed that rugose is only able to exhibit itself in the male sex,—females never show the character, even when homozygous. I now have a pure stock which gives 100 percent rugose males, but only normal (appearing) females.

The next mutant to appear, after rugose, was frayed (figures 3 and 6) —so called because the dorsal bands of pigment on the abdomen are frayed out or irregularly broken at the ends as they extend down the sides of the body. Accompanying this are various other modifications, any one of which might be used in deriving the name of the mutant. Almost the entire fly is affected in some way. The thoracic bristles are reduced to little more than hairs (compare figures 6 and 8); some of the bristles on the head are entirely gone; frequently the aristae, and sometimes the entire antennae are aborted or wanting; the wing veins are frequently broken and disarranged in various ways; and finally the development of the embryo is retarded several days so that in a mixed culture the frayed flies hatch out considerably later than their normal brothers and sisters. This mutant shows a greater variety of modifications than any other I have seen. It originated (September, 1916) in a mass culture containing yellow and normal-colored flies. Among the males in this culture were several, both yellow and brown, showing the frayed character. Two of these frayed males mated to unrelated females gave in F_1 normal males and females, and in F_2 277 normal females, 166 normal males,[2] and 106 frayed males.

The eighth and last character of the series, called hairy eye (figure 7) bears a marked superficial resemblance to rugose, but it lacks the un-

[2] Some of these were yellow.

usually light color of the latter and has a sprinkling of heavy black hairs over the eye surface, giving it a peculiar bushy appearance entirely wanting in rugose. Hairy was first observed (November, 1916) in a single male individual in culture number V745 (a female heterozygous for vesiculated, forked, yellow and glazed, by a normal male). This male was yellow, but showed none of the other three characters. When bred to a normal female he gave in F_1 only normal-eyed flies, and in F_2 normal females and four classes of males as follows: normal 37, hairy 28, yellow 22, yellow hairy 36; or 59 not hairy to 64 hairy, a typical result for a sex-linked recessive. Subsequent matings have indicated that hairy females are completely sterile, and consequently a pure stock cannot be maintained.

LINKAGE

In taking up the question of the linkage relations between these eight characters, it may be well at the outset to state that the general features of linkage in *Drosophila virilis* appear to be entirely comparable with those already well known in *D. ampelophila*. The amount of crossing over, and perhaps certain other details (to be taken up in another paper) differ somewhat in the two species, but there can be no question that in general the process is essentially the same in both. Single crossing over, double crossing over and triple crossing over occur in the sex-linked group of *D. virilis* in much the same manner as in that of *ampelophila;* and when plotted according to their linkage values the factors form a linear series showing the same general features as that of the sex-linked factors in *ampelophila*. The technique employed in this study, and the method of handling the data are, therefore, the same as those already well known from the work on *D. ampelophila* (see MORGAN, STURTEVANT, MULLER and BRIDGES 1915), and need little explanation. Whatever explanation is necessary will be given in its proper connection below.

In actual practice the linkage relations between the mutant characters were determined in the order in which the mutants arose, but in treating the data it is more convenient and more economical of space to take an arbitrary order. A glance at diagram 15 (page 131), showing the factors in their proper linear relations, will indicate the desirability of this. Several of the factors such as yellow and forked, yellow and glazed, frayed and glazed, etc., are so loosely linked as to show almost fifty percent of crossing over, and data involving these combinations alone are of little use in determining linkage values. It is more convenient, therefore, to consider each factor in connection with those adjacent to it; hence I shall take them up in serial order, beginning with those at the left end of the diagram.

Another arbitrary arrangement that I have adopted in the following pages is the separation of experiments involving only two pairs of characters (giving single crossovers) from those involving three or more pairs (giving multiple crossovers). It is more convenient to make use of the simpler experiments first in approximately determining the serial order of the various factors, and then to take up the more complex crosses to fill in the details. This puts the multiple crossover experiments in a convenient form for future reference, and at the same time makes it possible, by turning to the proper tables, to check up the conclusions derived from single crossover data.

SINGLE CROSSOVER EXPERIMENTS

Since the multiple crossover experiments give more accurate results than the singles, I shall not attempt to treat the latter extensively, but will merely cite a few cases to show the general relations beween the various factors when taken two at a time. In a few cases, where close linkage is involved, this method is fairly accurate, but as a rule it gives only a rough approximation to correct linkage values.

Experiment 1. Yellow and frayed

To determine the linkage between yellow and frayed, females heterozygous for yellow were mated to frayed males, giving daughters part of which were heterozygous for frayed and yellow. Counts were obtained from four females of this kind mated to normal males in pairs. As expected the daughters were all normal, and the sons were of four classes as shown in table 1. Out of 308 males 4 were crossovers, giving a crossover value of approximately 1 percent (shown in the summary of table 1). Expressing this in terms of units, yellow and frayed may be said to be approximately one unit apart, or very closely linked.

TABLE 1

Yellow × frayed.

Culture number	N	Y	F_r	$Y\,F_r$
V 810	0	59	36	1
V 811	1	106	68	2
V 814	0	45	25	0
V 847	0	34	21	0
Totals	1	154	150	3

Grand total 308

Summary

Y / F_r	$Y \mid F_r$
154	3
150	1
304	4

1 percent

Experiment 2. Yellow and vesiculated

Heterozygous females from matings between yellow and vesiculated gave normal females and four classes of males as shown in table 2. In this case there were 35 crossovers (23 percent) out of 151 flies, indicating that yellow and vesiculated are approximately 23 units apart.[3]

TABLE 2
Yellow × vesiculated.

Culture number	*N*	*V*	*Y*	*YV*
V 470	9	20	21	6
V 484	0	9	6	2
V 503	4	17	24	8
V 507	6	7	12	0
Totals	19	53	63	16

Grand total 151

Summary

Y / *V*	*Y V* / +
53	19
63	16
116	35

23 percent

Knowing the approximate linkage between yellow and frayed, and between yellow and vesiculated, it may be predicted that the serial order of the three factors is either frayed-yellow-vesiculated, or yellow-frayed-vesiculated, not yellow-vesiculated-frayed. Unfortunately the frayed stock was accidentally destroyed at this point in the experiments and the exact position of the factor (whether to the left or the right of yellow) must remain undetermined. (See experiment 14.)

Experiment 3. Magenta and forked

The characters magenta and forked were early found to be closely linked, and hence may be considered together in relation to those already treated. Table 3 shows the results obtained in F_2 from matings between

TABLE 3
Magenta × forked.

Culture number	*N*	*F*	*M*	*MF*
V 455	2	14	16	0
V 541	1	7	10	1
V 542	1	27	8	0
V 581	2	10	14	1
V 628	2	20	20	0
V 717	0	34	45	2
V 718	1	16	8	0
Totals	9	128	121	4

Grand total 262

Summary

M / *F*	*M F* / +
121	4
128	9
249	13

5 percent

[3] This is only a rough estimate based on very small numbers, and as shown by other experiments is probably five or six units too large.

magenta and forked. Out of 262 flies (males) 13, or approximately 5 percent, are crossovers, indicating that forked and magenta are about 5 units apart.

When tested with yellow, both magenta and forked show such a slight degree of linkage as to indicate that they are probably 50 or more units from that factor. This is shown in tables 4 and 5. When tested with

TABLE 4

Yellow × magenta.

Culture number	*N*	*M*	*Y*	*YM*
V 362	12	20	14	18
V 366	3	7	9	10
V 367	5	12	5	6
V 371	8	31	11	8
V 375	4	12	10	5
V 382	9	19	13	7
V 391	5	8	8	7
V 393	11	19	20	12
V 395	10	18	14	7
V 401	8	10	12	7
V 411	6	10	13	4
V 424	12	13	21	7
V 502	13	15	24	14
Totals	106	194	174	112

Grand total 586

Summary

$\frac{Y}{M}$	$\frac{Y\ M}{}$
174	112
194	106
368	218

37 percent

TABLE 5

Yellow × forked.

Culture number	*N*	*F*	*Y*	*YF*
V 462	14	13	17	4
V 473	13	7	17	9
V 476	10	19	24	10
V 492	16	11	19	5
V 493	5	6	11	5
V 498	12	14	29	6
V 501	10	5	6	9
V 509	8	5	17	5
V 510	7	4	8	3
Totals	95	84	148	56

Grand total 383

Summary

$\frac{Y}{F}$	$\frac{Y\ F}{}$
148	56
84	95
232	151

40 percent

vesiculated, forked gives nearly the same result as it did with yellow (table 7); but magenta shows a slight amount of linkage (table 6).

From these facts it is possible to conclude provisionally (confirmed by subsequent data) that the position of magenta and forked in the series is at the right of vesiculated. In other words the order is vesiculated-magenta-forked as shown in diagram 15 (page 131).

TABLE 6

Vesiculated × *magenta.*

Culture number	*N*	*M*	*V*	*MV*
V 445	16	26	19	5
V 479	12	23	19	8
V 487	8	13	11	5
V 505	7	6	7	0
V 607	5	5	2	0
V 659	10	22	25	9
Totals	58	95	83	27

Grand total 263

Summary

V / *M*	*V* *M*
83	27
75	58
178	85

32 percent

TABLE 7

(a) *Vesiculated* × *forked.*

Culture number	*N*	*F*	*V*	*VF*
V 466	9	16	9	7
V 471	6	7	8	7
V 478	11	10	15	6
V 481	17	6	13	8
V 620	19	19	29	12
Totals	62	58	74	40

Grand total 234

Summary

V / *F*	*V* *F*
58	62
74	40
132	102

(b) *Vesiculated forked* × *normal.*

Culture number	*N*	*F*	*V*	*VF*
V 531	41	17	18	13
V 534	8	2	4	6
V 539	34	17	14	18
V 592	13	8	14	4
V 593	53	24	32	31
V 614	24	9	12	11
V 618	21	4	10	14
V 630	47	12	14	31
Totals	241	93	118	128

Grand total 580

Grand total (a) and (b) 814

Summary

V F	*V* / *F*
128	118
241	93
369	211

102
211
313

37 percent

Hairy:—See experiment 10

Experiment 4. Forked and rugose

F_2 counts from forked by rugose, obtained in the usual manner, are summarized in table 8. The crossover value in this case is approximately 28 percent. This indicates that rugose must be either 28 units to the

TABLE 8

Forked × rugose.

Culture number	*N*	*F*	*R*	*FR*
V 611	9	13	23	0
V 626	28	36	36	10
V 631	4	13	8	0
V 638	10	11	18	3
V 653	8	11	5	2
V 725	1	14	17	6
Totals	60	98	107	21

Grand total 286

Summary

R / *F*	*R*, *F*
107	21
98	60
205	81

28 percent

right of forked, or else to the left between vesiculated and magenta. The latter possibility, however, is ruled out by the fact that vesiculated and rugose, when tested together, show almost no linkage. Similarly the multiple crossover results, where rugose is used, all agree in locating it well to the right of forked.

Rugose and glazed

Repeated attempts to cross these two mutants have all failed thus far, so I cannot give the precise location of their factors with respect to one another. But the fact that they give almost identical results in combination with other factors (see experiments 17 and 18) indicates that they are located close together; perhaps they are allelomorphic to one another.

MULTIPLE CROSSOVER EXPERIMENTS

In these experiments it will be possible to secure much more accurate determinations of linkage values than those given above. Both the serial order and the relative "positions" of the factors can thus be ascertained with a fair degree of accuracy. The method of determining the serial order is that already known from the work on *Drosophila ampelophila* (see MORGAN, STURTEVANT, MULLER and BRIDGES 1915). Since the same method is used throughout it need be described only once. This will be done in the first case given below. In the other cases the order will simply be stated, and it will be left to the reader to check up the statements by referring to the tables. The tables are so arranged as to make this a simple matter.

In order to facilitate comparison of the experiments as a whole I have constructed the graphic summaries shown in diagrams 1-15. Each diagram except the last represents a single experiment. The factors are indicated above the line, and are put in the positions determined by the crossover values of that particular experiment. The factor for yellow, being at one end of the series, is assigned an arbitrary position at zero, and the other factors are located on a line to the right of it. Whenever yellow is absent in an experiment vesiculated is used as a base and assigned the position given by the average of other experiments.

The last diagram is a rough average of the others. It is based largely upon diagrams seven to fifteen, because the preceding ones involve such long distances as to make them unreliable. It should be noted that the position of frayed (*Fr*) may perhaps be at the left of yellow, instead of at the right (see experiment 14), and that the location of rugose may not be exactly the same as that of glazed (see experiment 18).

THREE POINT EXPERIMENTS

Under this heading are grouped the experiments involving three pairs of factors. The first ones to be described are those including yellow; the later ones are those including only factors other than yellow.

Experiment 5. Yellow, magenta and forked

To determine the relations between these three factors yellow magenta males were mated to forked females and the F_1 females bred singly to their brothers or to other males. The sons of these females furnished the desired data.[4] Table 9 gives the results from seven such matings. As may be seen there are eight classes of male offspring. Of these the yellow magenta class and the forked class are non-crossovers; together they give 412, as shown in the summary of classes. The next smaller reciprocal classes are the magenta and the yellow forked. These total 248 as shown in the summary. Next in order of size are the normal and the yellow magenta forked classes, totalling 12 individuals. The remaining two classes, yellow and magenta forked, respectively, include only 5 flies. Assuming that the smallest group represents the double crossovers it is clear that the arrangement of the factors must be in the order yellow-magenta-forked. This being the case the pre-

[4] This method, with few exceptions, has been followed throughout all of the experiments described here. In some instances two or three females were put in the same bottle, but usually these gave about the same results as did single females.

ceding group (normal and yellow magenta forked) represents crossovers between magenta and forked, and the next preceding group represents crossovers between yellow and magenta. By adding the double crossover group to each of the others independently the total number of crossovers between yellow and magenta, and that between magenta and forked are obtained as shown in table 9. These are respectively 37 percent and 3 percent (in round numbers) of the total number of flies. By transposing percentages into units of distance, yellow and magenta are put 37 units apart and forked is put 3 units beyond magenta, as shown in diagram 1, page 131.

TABLE 9

Yellow magenta × forked.

Culture number	*N*	*M*	*F*	*MF*	*Y*	*YM*	*YF*	*YMF*
V 635	0	4	6	0	0	13	2	0
V 755	1	22	33	1	1	38	20	1
V 756	4	27	44	0	0	33	17	1
V 768	1	14	32	1	1	31	21	0
V 779	2	40	43	0	0	56	19	1
V 780	1	23	23	0	1	32	20	0
V 800	0	13	17	0	0	11	6	0
Totals	9	143	198	2	3	214	105	3

Grand total 677

Summary of classes

YM / *F*	*M* / *Y* *F*	*Y M F*	*M F* / *Y*
214	143	3	2
198	105	9	3
412	248	12	5

Summary of crossovers

Y–M	*M–F*
248	12
5	5
253	17
37%	3%

Experiment 6. Yellow, vesiculated and forked

In this experiment vesiculated females were mated to yellow forked males and the F_2 sons recorded as shown in table 10. Only 187 flies were obtained, and hence the results are subject to considerable error, but they are sufficient to show the relative positions of the factors concerned, and, as may be seen, they agree approximately with those from other experiments.

The crossover values in this case are 22 percent between yellow and vesiculated, and 32 percent between vesiculated and forked, or a total of 54 units between yellow and forked. As may be noted the 54 units here represent a wide deviation from the 40 units between yellow and forked in the preceding experiment. This is due, as will appear later, to the presence of at least one long "distance" in each case, giving opportunity for many undetectable double crossovers to occur. Especially is this true of the experiment involving yellow, magenta and forked, for yellow and magenta are in reality 50 or more units apart. Double crossing over here greatly reduces the apparent crossover value.

TABLE 10

Yellow forked × *vesiculated.*

Culture number	*N*	*F*	*V*	*FV*	*Y*	*YF*	*YV*	*YVF*	Total
V 642	1	2	17	1	6	12	3	1	43
V 644	0	3	10	6	8	5	2	2	36
V 658	3	3	8	4	2	5	2	1	28
V 680	4	1	17	5	4	4	1	0	36
V 683	0	1	4	1	4	5	0	2	17
V 697	2	4	9	1	2	6	3	0	27
Totals	10	14	65	18	26	37	11	6	187

Summary of classes

Y F / *V*	*Y*, *V* / *F*	*Y* / *V*, *F*	*Y*, *V*, *F*
29	5	7	2
15	5	14	2
13	5	6	4
21	2	9	4
9	1	5	2
15	7	3	2
102	25	44	16

Summary of crossovers

Y-V	*V-F*
25	44
16	16
41	60
22%	32%

Experiment 7. Yellow, vesiculated and magenta

This experiment differs little from the preceding, since magenta and forked are only a few units apart. Table 11 shows the results from the few counts obtained. Yellow and vesiculated in this case give a crossover value of 17 percent, as compared with 22 percent in the former experiment. Vesiculated and magenta give a value of about 30 percent, placing magenta 30 units beyond vesiculated. This is slightly less than the distance between vesiculated and forked, and harmonizes with the results given in the yellow magenta forked cross (experiment 5).

TABLE II

Yellow vesiculated magenta.

(a) Yellow × vesiculated magenta

Culture number	*N*	*M*	*V*	*VM*	*Y*	*YM*	*YV*	*YVM*	Total
V 512	3	1	5	6	10	2	4	1	32
V 518	1	1	7	9	13	3	3	0	37
Totals	4	2	12	15	23	5	7	1	69

Summary of classes

$\frac{Y}{VM}$	$\frac{\quad}{Y \mid VM}$	$\frac{Y \mid M}{V}$	$\frac{\quad \mid \quad M}{Y \mid V}$
23	4	5	2
15	1	12	7
38	5	17	9

(b) Yellow vesiculated × magenta

Culture number	*N*	*M*	*V*	*VM*	*Y*	*YM*	*YV*	*YVM*	Total
V 594	7	18	3	1	2	4	20	6	61
V 622	3	8	1	0	1	2	5	2	22
Totals	10	26	4	1	3	6	25	8	83

$\frac{YV}{M}$	$\frac{\quad \mid V}{Y \mid M}$	$\frac{YV \mid M}{\quad}$	$\frac{\quad \mid V \mid M}{Y}$
25	4	8	1
26	6	10	3
51	10	18	4

(c) Vesiculated × yellow magenta

Culture number	*N*	*M*	*V*	*VM*	*Y*	*YM*	*YV*	*YVM*	Total
V 619	0	3	11	7	2	11	1	0	35

Grand total 187

$\frac{V}{Y \; M}$	$\frac{Y \mid V}{M}$	$\frac{V \mid M}{Y}$	$\frac{\quad}{Y \mid V \mid M}$
11	1	7	0
11	3	2	0
22	4	9	0
111	19	44	13

Summary of crossovers

Y–V	*V–M*
19	44
13	13
32	57
17%	30%

Experiment 8. Yellow, forked and glazed

This experiment is of value mainly in showing the long "distances" between the respective factors used. The number of flies here (451) is sufficient to give fairly accurate results, so far as accurate results may be obtained from such a mating, but as may be seen (diagram 4) the crossover values—39 between yellow and forked, and 25 between forked and glazed—are so large as necessarily to be approximations only.

TABLE 12

Yellow glazed × *Forked*

Culture number	*N*	*F*	*G*	*GF*	*Y*	*YF*	*YG*	*YGF*	Total
V 488	7	6	7	2	7	1	14	0	44
V 499	4	3	3	2	5	4	11	4	36
V 524	3	15	13	9	8	9	12	5	74
V 529	1	6	6	0	2	4	4	0	23
V 536	1	4	5	0	3	1	6	0	20
V 540	3	2	3	1	8	4	8	0	29
V 543	3	5	8	0	3	2	8	0	29
V 564	5	7	3	1	6	4	7	0	33
V 571	0	7	6	0	2	5	10	3	33
V 583	0	3	1	0	3	4	7	0	18
V 649	6	26	22	0	6	15	37	0	112
Totals	33	84	77	15	53	53	124	12	451

Summary of classes

Y G / *F*	*G* / *Y* | *F*	*Y* / *F* | *G*	*Y* | *F* | *G*
20	8	9	7
14	7	7	8
27	22	17	8
10	10	2	1
10	6	3	1
10	7	9	3
13	10	3	3
14	7	7	5
17	11	2	3
10	6	3	0
63	37	6	6
208	131	68	45

Summary of crossovers

Y–F	*F–G*
131	68
45	45
176	113
39%	25%

Experiment 9. Yellow, vesiculated and glazed

Eight females from a cross of vesiculated female by yellow glazed male gave 579 male offspring as summarized in table 13. The crossover percentage between yellow and vesiculated is in this case 16, as compared with 17 and 22 in the preceding experiments. The percentage between vesiculated and glazed is 40,—i.e., practically no linkage. In reality the "distance" between these latter two is much greater, as shown by other experiments, but is concealed here by undetected double crossing over.

TABLE 13
Yellow glazed × vesiculated.

Culture number	*N*	*V*	*G*	*GV*	*Y*	*YV*	*YG*	*YGV*	Total
V 666	3	18	4	9	13	7	25	1	80
V 667	1	17	3	7	17	4	17	3	69
V 668	0	6	1	3	1	2	2	1	16
V 669	7	15	4	8	17	3	9	0	63
V 670	2	19	2	16	19	3	22	0	83
V 671	7	33	5	19	17	7	34	1	123
V 672	3	26	5	10	20	3	27	2	96
V 701	5	15	1	4	13	2	9	0	49
Totals	28	149	25	76	117	31	145	8	579

Summary of classes

Y G / *V*	+ *G* / *Y* + *V*	*Y* + / *V* + *G*	+ + + / *Y* *V* *G*
43	11	22	4
34	7	24	4
8	3	4	1
24	7	25	7
41	5	35	2
67	12	36	8
53	8	30	5
24	3	17	5
294	56	193	36

Summary of crossovers

Y–V	*V–G*
56	193
36	36
92	229
16%	40%

Experiment 10. Yellow, hairy and forked

Hairy has been tested in only two combinations. The first, with yellow and magenta, indicated that hairy was closely linked to magenta. Only 62 flies were secured in this mating, so the data are not given in detail. In the second case yellow hairy males were mated to forked females. This time 162 F_2 males were secured. They fall into only six classes, due to the entire absence of double crossover individuals (table 14). The missing classes are hairy forked and yellow, indicating that hairy lies to the left of forked and is very closely linked to it. Only five crossovers between hairy and forked are represented among the 162 flies. This would give hairy a position about 3 units from forked, or about half way between forked and magenta; and until further data are obtained it may provisionally be located here.

Experiment 11. Vesiculated, magenta and rugose

In this experiment, as in number 9, long distances are involved. Table 15 is a summary of the F_2 males from a cross of magenta female by vesiculated rugose male. The crossover percentage between vesiculated

TABLE 14

Yellow hairy × *forked.*

Culture number	*N*	*H*	*F*	*HF*	*Y*	*YH*	*YF*	*YHF*
V 1041	0	14	18	0	0	19	13	2
V 1042	2	17	20	0	0	35	21	1
Totals	2	31	38	0	0	54	34	3

Summary of classes

YH / *F*	*H* / *Y F*	*YH F*	*H F* / *Y*
54	31	3	0
38	34	2	0
92	65	5	0

Summary of crossovers

Y–H	*H–F*
65	5
40%	3%

TABLE 15

Magenta × *vesiculated rugose.*

Culture number	*N*	*M*	*R*	*RM*	*V*	*VM*	*VR*	*VRM*	Total
V 659	5	26	10	4	3	7	25	0	80
V 749	2	16	4	1	5	7	11	0	46
V 753	2	15	6	6	2	6	6	2	45
V 758	2	26	7	7	8	6	13	4	73
V 764	2	16	9	4	5	10	22	1	69
V 773	1	18	9	2	7	10	17	1	65
V 822	3	23	8	5	5	12	27	2	85
Totals	17	140	53	29	35	58	121	10	463

Summary of classes

M / *V R*	*V M* / *R*	*M R* / *V*	*V M R*
51	17	7	5
27	11	6	2
21	12	8	4
39	13	15	6
38	19	9	3
35	19	9	2
50	20	10	5
261	111	64	27

Summary of crossovers

V–M	*M–R*
111	64
27	27
138	91
29%	19%

and magenta is 29, as compared with 30 in experiment 7. That between magenta and rugose is 19. Both of these values are unduly small, even when allowance is made for double crossovers. Apparently crossing over was decreased in the whole experiment by some environmental or other cause undetected at the time.

Experiment 12. Vesiculated, magenta and forked

This experiment differs from the preceding in that forked takes the place of rugose. A forked female mated to vesiculated magenta males gave normal daughters whose male offspring are shown in table 16. The crossover values are 37 between vesiculated and magenta, as compared with 29 and 30 in preceding cases, and 5 between magenta and forked as compared with 3 and 5 in preceding cases. A much closer approxima-

TABLE 16

Forked × vesiculated magenta.

Culture number	*N*	*M*	*F*	*MF*	*V*	*VM*	*VF*	*VMF*	Total
V 646	1	9	5	1	0	13	3	0	32
V 657	2	12	20	1	0	16	5	0	56
V 676	1	4	3	0	0	14	4	0	26
V 679	0	7	5	0	0	10	6	2	30
V 715	0	8	1	0	0	8	5	0	22
V 716	0	12	19	0	0	24	15	3	73
V 722	1	14	17	2	1	34	15	1	85
V 723	2	22	20	0	0	28	9	0	81
V 724	1	7	11	0	0	15	10	1	45
V 728	0	5	12	0	0	9	3	0	29
V 732	2	6	7	1	0	6	3	0	25
Totals	10	106	120	5	1	177	78	7	504

Summary of classes

F / *VM*	*V* | *F* / *M*	*VM* | *F*	*V* / *M* | *F*
18	12	1	1
36	17	2	1
17	8	1	0
13	13	2	0
9	13	0	0
43	27	3	0
51	29	2	3
48	31	2	0
26	17	2	0
21	8	0	0
13	9	2	1
297	184	17	6

Summary of crossovers

V–M	*M–F*
184	17
6	6
190	23
37%	5%

tion to correct linkage values is evident in this experiment because the relatively short "distance" between magenta and forked makes it possible to detect most of the crossovers.

Experiment 13. Vesiculated, forked and rugose

In this experiment a vesiculated rugose female was mated to forked males. The F_2 male offspring are summarized in table 17. Although the count is small (163 flies) the relative values harmonize fairly well with those in other experiments. The crossover percentage between vesiculated and forked is about 38, in comparison to 42 (37 plus 5) in the preceding experiment and 32 in experiment 6. The percentage between forked and rugose is 27, the most accurate determination of this distance thus far made.

TABLE 17
Vesiculated forked × rugose.

Culture number	*N*	*F*	*R*	*FR*	*V*	*VF*	*VR*	*VFR*	Total
V 726	8	9	11	3	4	13	13	3	64
V 727	5	4	15	0	2	10	5	7	48
V 731	5	6	19	2	2	5	16	6	61
Totals	18	19	45	5	8	28	34	16	173

Summary of classes

$\frac{VF}{R}$	$\frac{F}{V\ R}$	$\frac{VF\ R}{}$	$\frac{F\ R}{V}$
24	22	11	7
25	9	12	2
24	22	11	4
73	53	34	13

Summary of crossovers

V–F	*F–R*
53	34
13	13
66	47
38%	27%

Experiment 14. Frayed, vesiculated and magenta

In the cross between yellow and frayed described above (experiment 1) it was shown that frayed is very closely linked to yellow. The present experiment summarizes the only other linkage data obtained from frayed before the stock was lost. It is of interest mainly in corroborating the other experiment as to the position of frayed.

Frayed males mated to vesiculated magenta females gave F_2 males as shown in table 18. According to these data the factor for frayed lies 18 units to the left of vesiculated. This is within one or two units of the position of yellow as given in the six experiments involving vesiculated and yellow. But since the value fluctuates more than two units in different experiments the figures do not determine whether frayed lies at the left or the right of yellow.

TABLE 18

Vesiculated magenta × *frayed.*

Culture number	*N*	*M*	*V*	*VM*	*Fr*	*FrM*	*FrV*	*FrVM*	Total
V 951	6	4	11	17	18	9	2	2	
V 953	6	3	13	29	34	21	2	4	
V 954	4	3	7	11	9	3	1	1	
V 955	7	3	5	20	20	14	6	2	
Totals	23	13	36	77	81	47	11	9	296

Summary of classes

VM / *Fr*	*Fr* | *VM*	*V* | / *Fr* | *M*	*Fr* | *V* | *M*
35	8	20	6
63	10	34	5
20	5	10	4
40	9	19	8
158	32	83	23

Summary of crossovers

Fr-V	*V-M*
32	83
23	23
55	106
18%	35%

FOUR POINT EXPERIMENTS

Under this heading are included the experiments involving four pairs of factors. These give sixteen classes of males in F_2—furnishing triple crossovers in addition to the doubles and singles of three point crosses. They are the most significant experiments of all, since they make it possible to break up the long "distances" into their shorter components and analyse them more accurately than in the two or three point crosses.

It will not be necessary, however, to consider each mating in detail. The methods are essentially the same as those used in three point crosses. In each case the heading of the table indicates how the cross was made, and the table includes only the F_2 males. The crossover value for any two factors is computed from the sum of single, double and triple crossovers involving the region between them.

Experiment 16. Yellow, vesiculated, magenta and forked

Alternate factors were introduced from the same side in this experiment by mating yellow magenta females to vesiculated forked males. The sons were all yellow magenta and the daughters normal. From the latter, sixteen classes of sons were obtained as summarized in table 19. By adding complementary classes eight groups are obtained as shown in the lower part of the table. The first of these is the non-crossover group; then follow the three single crossover groups, the three

TABLE 19

Yellow magenta × vesiculated forked.

Culture number	*N*	*M*	*F*	*V*	*MF*	*MV*	*VF*	*VM F*	*Y*	*YM*	*YF*	*YV*	*YM F*	*YV M*	*YV F*	*YV MF*	Totals
V 754	1	5	3	1	0	7	30	0	1	22	16	0	0	2	8	0	96
V 762	0	6	4	2	0	22	31	1	2	34	21	2	1	2	3	0	131
V 781	0	8	6	2	0	18	23	0	2	36	16	0	0	5	11	0	127
V 785	0	1	2	0	0	13	19	1	0	17	12	0	2	5	3	0	75
V 786	0	4	0	0	0	17	21	0	0	18	5	0	0	3	8	0	76
V 787	1	5	0	0	0	14	31	0	0	32	12	1	1	1	1	0	99
V 798	1	0	0	0	0	4	9	0	0	5	10	0	2	1	1	0	33
V 825	0	5	2	0	1	3	15	0	1	14	9	0	0	2	5	0	57
V 833	0	1	2	0	0	2	5	0	1	14	7	0	0	4	0	0	36
V 901	0	2	1	1	0	5	12	0	0	14	2	0	0	2	2	0	41
V 902	0	2	0	0	0	3	11	0	0	11	8	0	1	0	1	0	37
V 903	0	3	0	0	0	6	8	1	0	31	12	0	2	0	3	0	66
V 905	0	2	1	1	0	6	8	0	0	8	1	1	0	0	3	0	31
V 906	0	4	3	1	0	10	14	0	0	22	6	0	1	2	3	0	66
V 907	1	9	0	1	1	3	20	1	0	20	9	0	0	4	2	0	71
V 919	0	3	1	0	0	9	12	0	1	9	8	1	0	0	0	0	44
Totals	4	60	25	9	2	142	269	4	8	307	152	5	10	33	54	0	1086

Summary of classes

	Y M / V F	*M / Y VF*	*V M / Y F*	*Y M F / V*	*Y V M / F*	*Y V / M F*	*Y / V M F*	*Y V M F*
	307	60	142	10	33	5	8	0
	269	54	152	9	25	2	4	4
Totals	576	114	294	19	58	7	12	4

Summary of crossovers

	Y–V	*V–M*	*M–F*
	114	294	19
	58	58	7
	7	12	12
	4	4	4
Totals	183	368	42
	17%	34%	4%

double crossover groups and finally the triple crossover group. As may be seen no other arrangement of the factors than that given will account for the graduated size of the classes.

By determining the percentage of crossing over as in preceding cases the "distances" are given as follows: yellow to vesiculated 17 units; vesiculated to magenta 34 units; magenta to forked 4 units. How well these harmonize with other experiments may be seen by comparing diagram 11 with diagrams 2, 3 and 8.

Experiment 17. Yellow, vesiculated, forked and glazed

Table 20 and diagram 12 summarize the main features of this experiment. Vesiculated forked females were mated to yellow glazed males and the F_2 males recorded. These are in fifteen classes,—one expected triple crossover class (yellow vesiculated) being absent. Summarized in the same manner as before the crossover data are as follows: Non-crossovers 109; single crossovers between yellow and vesiculated 29;

TABLE 20

Yellow glazed × vesiculated forked.

Culture number	*N*	*V*	*F*	*G*	*VF*	*VG*	*FG*	*VFG*	*Y*	*YV*	*YF*	*YG*	*YVF*	*YVG*	*YFG*	*YFVG*	Total
V 719	1	3	2	7	15	10	0	5	12	0	19	14	4	1	5	1	99
V 721	2	6	2	2	18	8	0	4	6	0	5	18	4	0	4	2	81
V 721-2	0	5	3	3	17	8	1	2	7	0	9	9	6	2	1	0	73
V 745	1	4	0	2	7	5	0	0	7	0	6	11	1	0	1	0	45
Totals	4	18	7	14	57	31	1	11	32	0	39	52	15	3	11	3	298

Summary of classes

Y G / *VF*	*G* / *Y VF*	*Y F* / *V G*	*Y* / *VF G*	*Y V G* / *F*	*Y F G* / *V*	*Y VF G* /	*Y V* / *F G*
52	14	39	32	3	11	3	0
57	15	31	11	7	18	4	1
109	29	70	43	10	29	7	1

Summary of crossovers

	Y–V	*V–F*	*F–G*
	29	70	43
	10	10	29
	7	29	7
	1	1	1
	47	110	80
Totals	16%	37%	27%

singles between vesiculated and forked 70; singles between forked and glazed 43; doubles involving the regions yellow-vesiculated and vesiculated-forked 10; those involving the regions vesiculated-forked and forked-glazed 29; those involving the regions yellow-vesiculated and forked-glazed 7; triple crossovers 1. Adding the crossovers for each region independently and determining the percentages, the results are: 16 percent between yellow and vesiculated, 37 percent between vesiculated and forked, and 27 percent between forked and glazed—a very close approximation to corresponding regions in the preceding experiment.

Experiment 18. Yellow, vesiculated, forked and rugose

Except for the substitution of rugose in place of glazed this is a repetition of the preceding cross; and as seen in the summary (table 21) and

TABLE 21

Vesiculated forked × yellow rugose.

Culture number	*N*	*F*	*R*	*V*	*FR*	*VF*	*VR*	*VFR*	*Y*	*YF*	*YR*	*YV*	*YFR*	*YVF*	*YVR*	*YVFR*	Totals
V 783	2	5	1	7	0	16	9	4	14	9	24	4	5	1	0	1	102
V 784	1	0	5	5	1	12	13	5	6	15	13	0	3	0	1	1	81
V 803	4	5	5	5	0	22	23	7	13	15	28	3	1	8	3	0	142
V 804	0	6	2	6	1	14	4	2	10	10	9	0	1	5	1	1	72
V 819	0	2	5	5	1	16	15	8	11	16	25	1	4	6	3	1	119
V 820	6	1	4	1	0	19	11	0	9	9	22	2	4	1	1	1	91
V 826	0	1	3	6	0	11	3	4	7	11	9	3	0	2	1	1	62
V 831	1	0	3	1	0	3	3	2	1	1	12	0	0	3	0	0	30
Totals	14	20	28	36	3	113	81	32	71	86	142	13	18	26	10	6	699

Summary of classes

VF / *Y R*	*Y* | *VF* / *R*	*V* | *R* / *Y* | *F*	*VF* | *R* / *Y*	| | *F* / *Y V R*	*V* | | / *Y F R*	*Y* | *VF* | *R*	*Y* | *V* | | / *F R*
113	26	81	32	20	36	6	13
142	28	86	71	10	18	14	3
255	54	167	103	30	54	20	16

Summary of crossovers

Y–V	*V–F*	*F·R*
54	167	103
30	30	54
20	54	20
16	16	16
120	267	193
17%	38%	27%

in diagram 13, the respective percentages are almost exactly the same. These two experiments leave little doubt that glazed and rugose are very closely linked.

Experiment 19. Yellow, vesiculated, magenta and rugose

In this experiment magenta was substituted for forked, and the factors were introduced in different combinations from those given in the last case. Yellow magenta females were mated to vesiculated rugose males and the grandsons recorded in the usual manner (table 22). The crossover values are: 20 percent between yellow and vesiculated, 29 percent between vesiculated and magenta, and 29 percent between magenta and rugose. Each of these deviates two or three percent from what

TABLE 22

Yellow magenta × vesiculated rugose.

Culture number	*N*	*M*	*R*	*V*	*MR*	*MV*	*RV*	*RMV*	*Y*	*MY*	*RY*	*VY*	*RMY*	*VMY*	*RYV*	*VMRY*	Total
V 909	0	2	1	6	5	7	15	2	3	20	11	1	7	1	5	3	89
V 910	0	3	1	3	0	0	7	1	2	10	4	1	4	0	1	0	37
V 911	0	5	4	9	5	12	17	3	5	23	14	3	5	2	6	1	114
V 912	0	2	0	3	1	4	8	4	4	11	7	0	3	1	5	1	54
V 914	1	5	0	5	0	2	0	0	1	14	0	2	1	0	0	0	31
V 920	0	1	0	7	0	1	10	0	2	9	2	0	2	0	2	0	36
Totals	1	18	6	33	11	26	57	10	17	87	38	7	22	4	19	5	361

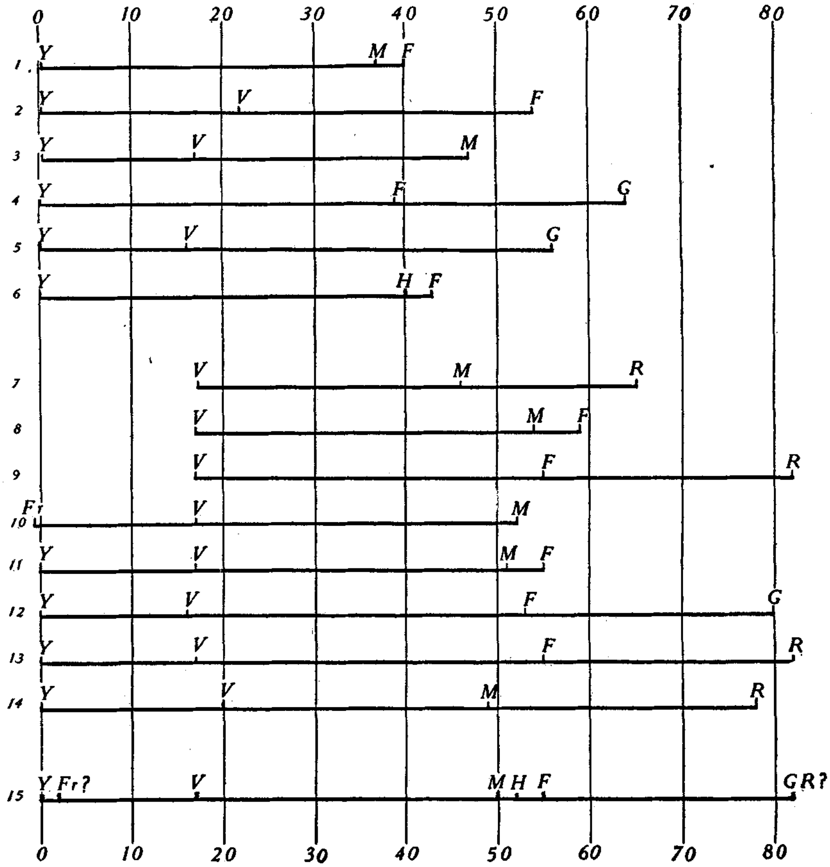
Summary of classes

	Y M / *V R*	*M* / *Y V R*	*Y R* / *V M*	*Y V M* / *R*	*Y M R* / *V*	*Y V* / *M R*	*Y* / *V M R*	*Y V M R*
	87	18	38	4	22	7	10	5
	57	19	26	6	33	11	17	1
Totals	144	37	64	10	55	18	27	6

Summary of crossovers

	Y-V	*V-M*	*M-R*
	37	64	55
	10	10	18
	18	27	27
	6	6	6
Totals	71	107	106
	20%	29%	29%

might be anticipated upon the basis of preceding experiments; but they show the same general features (diagram 14).



DIAGRAMS 1-15.—The diagrams represent crossover values (percentages) in terms of distance along a straight line. Numbers 1 to 14 summarize individual experiments. Number 15 is an approximate average of the others.

The position of frayed (*Fr*) is assigned mainly on the basis of an experiment (number 1) not included in the diagrams, and may perhaps be on the wrong side of yellow. Likewise the position of rugose (*R*) is not definitely known to coincide with that of glazed, but is known to be close to it.

DISCUSSION

It is not my purpose here to enter into a detailed discussion of linkage in *D. virilis* as compared with that in *D. ampelophila*. The general re-

[5] It may be noted in this connection that in *D. virilis*, just as in *D. ampelophila*, no mutant factors have been found in the Y chromosome of the male, and no crossing over between X and Y has been detected. Cytologically the X and Y chromosomes look very much alike (METZ 1916b).

semblance between the two is obvious from what has preceded,[5] and the work has scarcely progressed far enough to allow of more specific deductions; but there are two features brought out by the experiments that merit especial notice at this time.

The most interesting of these, in view of its possible significance, is the fact that two of the above described mutant characters in *virilis* bear a marked resemblance to two characters in *ampelophila*, and that the factors for these characters appear to occupy the same relative positions in the linkage group (chromosome). The two characters are yellow and forked. I have already called attention to the morphological resemblance they bear to their namesakes in *ampelophila* (METZ 1916 c, p. 431), and may confine myself here to a comparison of their linkage relations.

MORGAN and BRIDGES (1915) in summarizing the data on thirty-two sex-linked factors in *Drosophila ampelophila*, show that the factor for yellow is located at one extreme end of the linkage group. This they call the zero point. The factor for forked they locate 56.5 units to the right of this, or almost at the other end of the group (see their diagram 1, p. 22).

Now comparing with these the positions of yellow and forked in *virilis*, as given above, the resemblance is seen at a glance. Yellow in *virilis*, as in *ampelophila*, is at one end of the group, and forked falls about 55 units from it. The conclusion suggests itself at once that the two species correspond in respect to this much of their chromosomal organization. This particular case may, of course, be due merely to a coincidence, but if so it is remarkable that there should be such an agreement not only between the characters, but between the locations of the factors. To be sure this latter correspondence rests largely upon the terminal position of yellow in both species; and since I have studied only eight factors (sex-linked) in *virilis*, it is possible that some may be found later having loci well to the left of yellow, and removing the latter from its present terminal or sub-terminal position. On this point nothing can be said at present except that the linkage group in *virilis* is already longer (as measured by the actual amount of crossing over) than that in *ampelophila*. In *ampelophila* the sex chromosome map compiled by MORGAN and BRIDGES (1915) is only 66.2 units long, while that in *virilis*, given by the eight factors already studied, is 82 units.

Unfortunately yellow and forked are the only sex-linked characters in *virilis* that bear a striking resemblance to any in *ampelophila*, and the comparison between the species cannot be extended to include a series of factors. Hence the evidence is by no means conclusive. I am now endeavoring to get enough additional mutants to throw more light on this point.

The second feature to which I wish to call attention is the large amount of crossing over apparent in *virilis* as compared with *ampelophila*. Whether this difference is sufficient to vitiate the comparison of linkage values given above is not clear; but in any case there is a noticeable difference as shown by the fact just mentioned, that a crossover distance of 82 units is given by the six factors yellow to glazed (leaving frayed and rugose out of account). If the 82 units represent chromosome length, then either we are dealing here with a longer chromosomal region than in *ampelophila*, or else crossing over is materially greater per unit of actual (not calculated) chromosome length. Cytologically the sex chromosomes of the two species look very much alike.[6] Those of *virilis* are perhaps a little longer, because the fly is larger, and corresponding cells (e.g., oogonia) are somewhat larger; but it is very doubtful whether this has anything to do with the difference in crossover distances.

In *ampelophila* it has been shown that linkage (crossing over) is not an absolutely fixed characteristic but may be markedly affected in different ways. For instance changes in temperature alter the amount of crossing over (PLOUGH 1917); the second brood of a female may give fewer crossovers than the first (BRIDGES 1915); and definite heritable factors have been found by STURTEVANT to lower the crossover values (MULLER 1916, p. 213). It would not be surprising, then, if a different species were found to give a different amount of crossing over, entirely irrespective of chromosome length. This I believe to be the case in *virilis*.

Judging from the data already in hand the calculated length of the distance from yellow to glazed, to say nothing of possible distances beyond them, will probably reach ninety or more units when more factors are located in this region. It is well known that the most accurate evaluation of a long distance is obtained by adding together its component short distances, and in *virilis* three long distances intervene between yellow and glazed without any available factors to break them up. These are the regions, yellow to vesiculated (17 units), vesiculated to magenta (33 units), and forked to glazed (27 units). Each of them affords

[6] Compare METZ 1916 b, plate 1, figures 2 and 19. The lower pair of chromosomes is the sex chromosome pair in figure 19.

space for numerous double crossovers, and consequently they ought to be increased by several units when more factors are studied.

It might seem at first glance that this greater frequency of crossing over in *virilis* removes the significance from the parallelism that I have just noted between the factorial loci of yellow and forked in the two species. This is not necessarily so, however. If the factors do correspond in the two species it would be expected that those in *virilis* would be more loosely linked than those in *ampelophila,* because of the greater amount of crossing over in the former. The actual values do not show this at present; but, as I have pointed out in a preceding paragraph, the calculated distance between yellow and forked in *virilis* is sure to be increased when additional factors are located in this region. It will probably become more than sixty units, so that the relative positions of the two factors may not differ greatly from those in *ampelophila.*

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