THE LINEAR ARRANGEMENT OF SIX SEX-LINKED FACTORS IN DROSOPHILA, AS SHOWN BY THEIR MODE OF ASSOCIATION

A. H. STURTEVANT

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INTRODUCTION

In 1998, with genome projects routinely producing detailed genetics maps of mice and men and every other sort of organism, it can be difficult to imagine a time when there were no genetic maps. The idea that individual genes occupy regular positions on chromosomes was one of the great insights of early genetics, and the very first genetic map was published in 1913 by Alfred H. Sturtevant, who was working on fruit flies in the laboratory of Thomas H. Morgan at Columbia University.

Sturtevant is now well known as one of the most important early pioneers in genetic research. However, at the time he produced the first map, he was an undergraduate. Many years later, Sturtevant (*A History of Genetics*) described how an undergraduate came to be crucially involved in establishing the very foundations of classical genetics:

> In 1909, the only time during his twenty–four years at Columbia, Morgan gave the opening lectures in the undergraduate course in beginning zoology. It so happened that C. B. Bridges and I were both in the class. While genetics was not mentioned, we were both attracted to Morgan and were fortunate enough, though both still undergraduates, to be given desks in his laboratory the following year (1910–1911). The possibilities of the genetic study of Drosophila were then just beginning to be apparent; we were at the right place at the right time. … In the latter part of 1911, in conversation with Morgan … I suddenly realized that the variations in strength of linkage, already attributed by Morgan to differences in the spatial separation of the genes, offered the possibility of determining sequences in the linear dimension of a chromosome. I went home and spent most of the night (to the neglect of my undergraduate homework) in producing the first chromosome map, which included the sex–linked genes *y*, *w*, *v*, *m*, and *r*, in the order and approximately the relative spacing that they still appear on the standard maps (Sturtevant, 1913).

This 1913 paper not only produced the first genetic map, with all of its genes in their correct position, but it also clearly laid out the logic for genetic mapping. Sturtevant noted that map "distance", as he calculated it, was not a measurement of physical distance but rather was some joint function of length and strength over a region of chromosome. He also correctly analyzed the effects of multiple cross-overs on the measurement of map distances (see the section "Double Crossing Over" beginning on page 8), and he noted that the occurrence of one cross over seems to inhibit the occurrence of additional cross overs (a phenomenon now know as **INTERFERENCE**):

> Double crossing over does then occur, but it is to be noted that the occurrence of the break between B and CO tends to prevent that between CO and R (or vice versa). Thus where B and CO did not separate, the gametic ratio for CO and R was about 1 to 2, but in the cases where B and CO did separate it was about 1 to 6.5. Three similar cases from my own results, though done on a smaller scale, are given in the table at the end of this paper. The results are represented in Tables 5, 6, and 7.

Although the paper is remarkable for the depth and clarity of its analysis, some aspects of its presentation may be difficult for the modern reader. In particular, Table 8 can seem impenetrable at first glance, but it is really just a detailed presentation of the raw progeny data behind the proportion-of-cross-over data in Table 2.

For example, Table 2 has an entry that reads

$$\text{BO} \qquad\qquad \frac{2}{373} \qquad\qquad 0.5$$

and the corresponding Table 8 entry is

BO.	P_1:	gray-eosin ♀ × yellow-red ♂			
	F_1:	gray-red ♀ × gray-eosin ♂			
F_2:	♀ ♀,	g.r. 241,	g.e. 196		
	♂ ♂,	g.r. 0,	g.e. 176,	y.r. 195,	y.e. 2
		Proportion of crossovers,		$\frac{2}{373}$	

The Table 8 entry shows that the proportion of crossovers between factors B and O were obtained by crossing parental (P_1) gray-eosin females with yellow-red males, then crossing the gray-red females and the gray-eosin males of the F_1 to obtain an F_2. The different male phenotypes in the F_2 were then counted to allow the determination of the actual proportion-of-crossovers value for the experiment.

Since crossing over can only occur in females (the males have, after all, only one X chromosome), only the male progeny of the F_2 are used in determining the proportion of crossovers. Phenotypic combinations that occur in F_2 males, but were not present in the parental generation, represent cross-over events. The male F_2 data* for this cross are g.r. = 0, g.e. = 176, y.r. = 195, and y.e. = 2.

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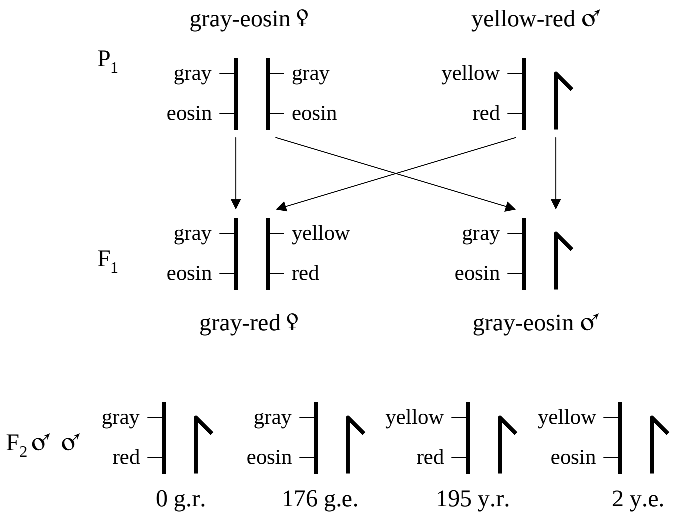
* Where g.r = gray-red, g.e.= gray-eosin, y.r. = yellow-red, and y.e. = yellow eosin.

An illustration of the cross, showing the chromosomes, illustrates the logic:



There are a total of 373 male progeny and, of them, only 2 represent cross-over events. Thus the proportion of crossovers is 2/373, or 0.5.

In the paper, Sturtevant offers in Table 1 his own explanation of the logic behind his analysis, but the utility of that explanation for the modern reader is somewhat limited by the use of outdated symbology and significant reduced by the presence of a mathematical error. In that table, and carried throughout the paper, Sturtevant shows a total of 405 male progeny for that cross when in fact the individual values add to 458. Sturtevant himself noted this error in a later reprinted collection of his papers (Lewis, E. B. [ed.] 1961. *Genetics and Evolution: Selected Papers of A. H. Sturtevant.* San Francisco: W. H. Freeman and Co.).

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HISTORICAL

The parallel between the behavior of the chromosomes in reduction and that of Mendelian factors in segregation was first pointed out by Sutton (1902) though earlier in the same year Boveri (1902) had referred to a possible connection. In this paper and others Boveri brought forward considerable evidence from the field of experimental embryology indicating that the chromosomes play an important role in development and inheritance. The first attempt at connecting any given somatic character with a definite chromosome came with McClung's (1902) suggestion that the accessory chromosome is a sex-determiner. Stevens (1905) and Wilson (1905) verified this by showing that in numerous forms there is a sex chromosome, present in all the eggs and in the female-producing sperm, but absent, or represented by a smaller homologue, in the male-producing sperm. A further step was made when Morgan (1910) showed that the factor for color in the eyes of the fly *Drosophila ampelophila* follows the distribution of the sex chromosome already found in the same species by Stevens (1908). Later, on the appearance of a sex-linked wing mutation in *Drosophila*, Morgan (1910a, 1911) was able to make clear a new point. By crossing white-eyed, long-winged flies to those with red eyes and rudimentary wings (the new sex-linked character) he obtained, in F_2, white-eyed, rudimentary-winged flies. This could happen only if "crossing over" is possible; which means, on the assumption that both of these factors are in the sex chromosomes, that an interchange of materials between homologous chromosomes occurs (in the female only, since the male has only one sex chromosome). A point not noticed at this time came out later in connection with other sex-linked factors in Drosophila (Morgan 1911d). It became evident that some of the sex-linked factors are associated, *i.e.*, that crossing over does not occur freely between some factors, as shown by the fact that the combinations present in the F_1 flies are much more frequent in F_2 than are new combinations of the same characters. This means, on the chromosome view, that the chromosomes, or at least certain segments of them, are more likely to remain intact during reduction than they are to interchange materials.* On the basis of these facts Morgan (1911c, 1911d) has made a suggestion as to the physical basis of coupling. He uses Janssens' (1909) chiasmatype hypothesis as a mechanism. As he expresses it (Morgan 1911c):

> If the materials that represent these factors are contained in the chromosomes, and if those that "couple" be near together in a linear series, then when the parental pairs (in the heterozygote) conjugate like regions will stand opposed. There is good evidence to support the view that during the strepsinema stage homologous chromosomes twist around each other, but when the chromosomes separate (split) the split is in a single plane, as maintained by Janssens. In consequence, the original materials will, for short distances, be more likely to fall on the same side of the split, while remoter regions will be as likely to fall on the same side as the last, as on the opposite side. In consequence, we find coupling in certain characters, and little or no evidence at all of coupling in other characters, the difference depending on the linear distance apart of the chromosomal materials that represent the factors. Such an explanation will account for all the many phenomena that I have observed and will explain equally, I think, the other cases so far described. The results are a simple mechanical result of the location of the materials in the chromosomes, and of the method of union of homologous chromosomes, and the proportions that result are not so much the expression of a numerical system as of the relative location of the factors in the chromosomes.

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* It is interesting to read, in this connection, Lock's (1906, p. 248-253) discussion of the matter.

SCOPE OF THIS INVESTIGATION

It would seem, if this hypothesis be correct, that the proportion of "crossovers" could be used as an index of the distance between any two factors. Then by determining the distances (in the above sense) between A and B and between B and C, one should be able to predict AC. For, if proportion of crossovers really represents distance, AC must be approximately, either AB plus BC, or AB minus BC, and not any intermediate value. From purely mathematical considerations, however, the sum and the difference of the proportion of crossovers between A and B and those between B and C are only limiting values for the proportion of crossovers between A and C. By using several pairs of factors one should be able to apply this test in several cases. Furthermore, experiments involving three or more sex-linked allelomorphic pairs together should furnish another and perhaps more crucial test of the view. The present paper is a preliminary report of the investigation of these matters.

I wish to thank Dr. Morgan for his kindness in furnishing me with material for this investigation, and for his encouragement and the suggestions he has offered during the progress of the work. I have also been greatly helped by numerous discussions of the theoretical side of the matter with Messrs. H. J. Muller, E. Altenburg, C. B. Bridges, and others. Mr. Muller's suggestions have been especially helpful during the actual preparation of the paper.

THE SIX FACTORS CONCERNED

In this paper I shall treat of six sex-linked factors and their interrelationships. These factors I shall discuss in the order in which they seem to be arranged.

B stands for the black factor. Flies recessive with respect to it (b) have yellow body color. The factor was first described and its inheritance given by Morgan (1911a).

C is a factor which allows color to appear in the eyes. The white-eyed fly (first described by Morgan 1910) is now known to be always recessive with respect both to C and to the next factor.

O. Flies recessive with respect to O (o) have eosin eyes. The relation between C and O has been explained by Morgan in a paper now in print and about to appear in the Proceedings of the Academy of Natural Sciences in Philadelphia.

P. Flies with p have vermilion eyes instead of the ordinary red (Morgan 1911d).

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R. This and the next factor both affect the wings. The normal wing is RM. The rM wing is known as miniature, the Rm as rudimentary, and the rm as rudimentary-miniature. This factor R is the one designated L by Morgan (1911d) and Morgan and Cattell (1912). The L of Morgan's earlier paper (1911) was the next factor.

M. This has been discussed above, under R. The miniature and rudimentary wings are described by Morgan (1911a) .

The relative position of these factors is

$$\mathrm{B},\quad \frac{\mathrm{C}}{\mathrm{O}},\quad \mathrm{P},\quad \mathrm{R},\quad \mathrm{M}$$

C and O are placed at the same point because they are completely linked. Thousands of flies had been raised from the cross CO (red) by co (white) before it was known that there were two factors concerned. The discovery was finally made because of a mutation and not through any crossing over. It is obvious, then, that unless coupling strength be variable, the same gametic ratio must be obtained whether, in connection with other allelomorphic pairs, one uses CO (red) as against co (white), Co (eosin) against co (white), or CO (red) against Co (eosin) (the cO combination is not known).

METHOD OF CALCULATING STRENGTH OF ASSOCIATION

In order to illustrate the method used for calculating the gametic ratio I shall use the factors P and M. The cross used in this case was, long-winged, vermilion-eyed female by rudimentary-winged, red-eyed male. The analysis and results are seen in Table 1.

It is of course obvious from the figures that there is something peculiar about the rudimentary-winged flies, since they appear in far too small numbers. This point need not detain us here, as it always comes up in connection with rudimentary crosses, and is being investigated by Morgan. The point of interest at present is the linkage. In the F_2 generation the original combinations, red-rudimentary and vermilion-long, are much more frequent in the males (allowing for the low viability of rudimentary) than are the two new or crossover combinations, red-long and vermilion-rudimentary. It is obvious from the analysis that no evidence of association can be found in the females, since the M present in all female-producing sperm masks m when it occurs. But the ratio of crossovers in the gametes is given without complication by the F_2 males, since the male-producing sperm of the F_1 male bore no sex-linked genes. There are in this case 349 males in the noncrossover classes and 109 in the crossovers. The method which has seemed most satisfactory for expressing the relative position of factors, on the theory proposed in the beginning of this paper, is as follows. The unit of “distance” is taken as a portion of the chromosome of such length that, on the average, one crossover will occur in it out of every 100 gametes formed. That is, percent of crossovers is used as an index of distance. In the case of P and M there occurred 109 crossovers in 405 gametes, a ratio of 26.9 in 100; 26.9, the percent of crossovers, is considered as the “distance” between P and M.

Table 1

	Long-vermilion Rudimentary-red	♀ — MpX MpX ♂ — mPX
F$_1$	MpX mPX MpX	— long-red ♀ — long-vermilion ♂
Gametes F$_1$	Eggs Sperm	— MPX mPX MpX mpX — MpX
F$_2$	MPX MpX mPX MpX	— long-red ♀ — 451
	MpX MpX mpX MpX	— long-vermilion ♀ — 417
	MPX	— long-red ♂ — 105
	mPX	— rudimentary-red ♂ — 33
	MpX	— long-vermilion ♂ — 316
	mpX	— rudimentary-vermilion ♂ — 4

THE LINEAR ARRANGEMENT OF THE FACTORS

Table 2 shows the proportion of crossovers in those cases which have been worked out. The detailed results of the crosses involved are given at the end of this paper. The 16287 cases of B and CO are from Dexter (1912). Inasmuch as C and O are completely linked I have added the numbers for C, for O, and for C and O taken together, giving the total results in the lines beginning (C, O) P, B (C, O), *etc.*, and have used these figures, instead of the individual C, O, or CO results, in my calculations. The fractions in the column marked “proportion of crossovers” represent the number of crossovers (numerator) to total available gametes (denominator).

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As will be explained later, one is more likely to obtain accurate figures for distances if those distances are short, *i.e.*, if the association is strong. For this reason I shall, in so far as possible, use the percent of crossovers between adjacent points in mapping out the distances between the various factors. Thus, B (C, O), (C, O) P, PR, and PM form the basis of Diagram 1. The figures on the diagram represent calculated distances from B.

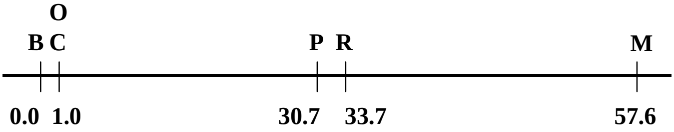

Diagram 1

Of course there is no knowing whether or not these distances as drawn represent the actual relative spatial distances apart of the factors. Thus the distance CP may in reality be shorter than the distance BC, but what we do know is that a break is far more likely to come between C and P than between B and C. Hence, either CP is a long space, or else it is for some reason a weak one. The point I wish to make here is that we have no means of knowing that the chromosomes are of uniform strength, and if there are strong or weak places, then that will prevent our diagram from representing actual relative distances — but, I think, will not detract from its value as a diagram.

Just how far our theory stands the test is shown by Table 3, giving observed percent of crossovers, and distances as calculated from the figures given in the diagram of the chromosome. Table 3 includes all pairs of factors given in Table 2 but not used in the preparation of the diagram.

It will be noticed at once that the long distances, BM, and (C, O) M, give smaller percent of crossovers, than the calculation calls for. This is a point which was to be expected, and will be discussed later. For the present we may dismiss it with the statement that it is probably due to the occurrence of two breaks in the same chromosome, or "double crossing over." But in the case of the shorter distances the correspondence with expectation is perhaps as close as was to be expected with the small numbers that are available. Thus, BP is 3.2 less than BR, the difference expected being 3.0. (C, O) R is less than BR by 1.8 instead of by 1.0. It has actually been found possible to predict the strength of association between two factors by this method, fair approximations having been given for BR and for certain combinations involving factors not treated in this paper, before the crosses were made.

Table 2

Factors concerned	Proportion of crossovers	Percent of crossovers
BCO	$\frac{193}{16287}$	1.2
BO	$\frac{2}{373}$	0.5
BP	$\frac{1464}{4551}$	32.2
BR	$\frac{115}{324}$	35.5
BM	$\frac{260}{693}$	37.6
COP	$\frac{224}{748}$	30.0
COR	$\frac{1643}{4749}$	34.6
COM	$\frac{76}{161}$	47.2
OP	$\frac{247}{836}$	29.4
OR	$\frac{183}{538}$	34.0
OM	$\frac{218}{404}$	54.0
CR	$\frac{236}{829}$	28.5
CM	$\frac{112}{333}$	33.6
B(C, O)	$\frac{214}{21736}$	1.0
(C, O) P	$\frac{471}{1584}$	29.7
(C, O) R	$\frac{2062}{6116}$	33.7
(C, O) M	$\frac{406}{898}$	45.2
PR	$\frac{17}{573}$	3.0
PM	$\frac{109}{405}$	26.9

Table 3

Factors	Calculated distance	Observed per cent of crossovers
BP	30.7	32.2
BR	33.7	35.5
BM	57.6	37.6
(C, O) R	32.7	33.7
(C, O) M	56.6	45.2

DOUBLE CROSSING OVER

On the chiasmatype hypothesis it will sometimes happen, as shown by Dexter (1912) and intimated by Morgan (1911d) that a section of, say, maternal chromosome will come to have paternal elements at both ends, and perhaps more maternal segments beyond these. Now if this can happen it introduces a complication into the results. Thus, if a break occurs between B and P, and another between P and M, then, unless we can follow P also, there will be no evidence of crossing over between B and M, and the fly hatched from the resulting gamete will be placed in the noncrossover class, though in reality he represents two crossovers. In order to see if double crossing over really does occur it is necessary to use three or more sex-linked allelomorphic pairs in the same experiment. Such cases have been reported by Morgan (1911d) and Morgan and Cattell (1912) for the factors B, CO, and R. They made such crosses as long-gray-red by miniature-yellow-white, and long-yellow-red by miniature-gray-white, *etc*. The details and analyses are given in the original papers, and for our present purpose it is only the flies that are available for observations on double crossing over that are of interest. Table 4 gives a graphical representation of what happened in the 10495 cases.

Double crossing over does then occur, but it is to be noted that the occurrence of the break between B and CO tends to prevent that between CO and R (or vice versa). Thus where B and CO did not separate, the gametic ratio for CO and R was about 1 to 2, but in the cases where B and CO did separate it was about 1 to 6.5. Three similar cases from my own results, though done on a smaller scale, are given in the table at the end of this paper. The results are represented in Tables 5, 6, and 7.

Table 4

No crossing over	Single crossing over		Double crossing over
B CO R	B CO R	B CO R	B CO R
6972	3454	60	9

Table 5

No crossing over	Single crossing over		Double crossing over
O P R	O P R	O P R	O P R
194	102	11	1

Table 6

No crossing over	Single crossing over		Double crossing over
B O M	B O M	B O M	B O M
278	160	1	0

Table 7

B O P R	B O P R	B O P R	B O P R	B O P R	B O P R	B O P R	B O P R
393	203	19	6	2	1	1	0

It will be noted that here also the evidence, so far as it goes, indicated that the occurrence of one crossover makes another one less likely to occur in the same gamete. In the case of BOPR there was an

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opportunity for triple crossing over, but it did not occur. Of course, on the view here presented there is no reason why it should not occur, if enough flies were raised. An examination of the figures will show that it was not to be expected in such small numbers as are here given. So far as I know there is, at present, no evidence that triple crossing over takes place, but it seems highly probable that it will be shown to occur.*

Unfortunately, in none of the four cases given above are two comparatively long distances involved, and in only one are there enough figures to form a fair basis for calculation, so that it seems as yet hardly possible to determine how much effect double crossing over has in pulling down the observed percent of crossovers in the case of BM and (C, O) M. Whether or not this effect is partly counter-balanced by triple crossing over must also remain unsettled as yet. Work now under way should furnish answers to both these questions.

Table 8

(The meaning of the phrase ‘proportion of crossovers’ is given on pp. 5-6)

BO.	P_1:	gray-eosin ♀ × yellow-red ♂			
	F_1:	gray-red ♀ × gray-eosin ♂			
F_2:	♀ ♀,	g.r. 241,	g.e. 196		
	♂ ♂,	g.r. 0,	g.e. 176,	y.r. 195,	y.e. 2
		Proportion of crossovers,		$\frac{2}{373}$	
BP.	P_1:	gray-red ♀ × yellow-vermilion ♂			
	F_1:	gray-red ♀ × gray-red ♂			
F_2:	♀ ♀,	g.r. 98;			
	♂ ♂,	g.r. 59,	g.v. 16,	y.r. 24,	y.v. 33
	Back cross, F_1 gray-red ♀ ♀ from above × yellow-vermilion ♂ ♂				
F_2:	♀ ♀	g.r. 31,	g.v. 11,	y.r. 12,	y.v. 41
	♂ ♂	g.r. 23,	g.v. 13,	y.r. 8,	y.v. 21
	P_1:	gray-vermilion ♀ × yellow-red ♂			
	F_1:	gray-red ♀ × gray-vermilion ♂			
F_2:	♀ ♀,	g.r. 199,	g.v. 182		
	♂ ♂	g.r. 54,	g.v. 149,	y.r. 119,	y.v. 41
	P_1:	yellow-vermilion ♀ × gray-red ♂			
	F_1:	gray-red ♀ × yellow-vermilion ♂			
F_2:	♀ ♀,	g.r. 472,	g.v. 240,	y.r. 213,	y.v. 414
	♂ ♂	g.r. 385,	g.v. 186,	y.r. 189,	y.v. 324

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* A case of triple crossing over within the distance CR was observed after this paper went to press.

Table 8 *(continued)*

	P_1:	gray-vermilion × yellow-red (sexes not recorded)			
	F_1:	gray-red ♀ ♀. These were mated to yellow-vermilion ♂ ♂ of other stock			
F_2:	♀ ♀	g.r. 50,	g.v. 96,	y.r. 68,	y.v. 41
	♂ ♂	g.r. 44,	g.v. 105,	y.r. 86,	y.v. 47
Proportion of crossovers, adding from BOPR (below), $\frac{1464}{4551}$					
BR.	P_1:	miniature-yellow ♀ × long-gray ♂			
	F_1:	long-gray ♀ × miniature-yellow ♂			
F_2:	♀ ♀	l.g. 14,	l.y. 2,	m.g. 7,	m.y. 6
	♂ ♂	l.g. 10,	l.y. 1,	m.g. 6,	m.y. 8
	P_1:	long-yellow ♀ × miniature-gray ♂			
	F_1:	long-gray ♀ × long-yellow ♂			
F_2:	♀ ♀,	l.g. 148,	l.y. 130		
	♂ ♂	l.g. 51,	l.y. 82,	m.g. 89,	m.y. 48
		Proportion of crossovers,		$\frac{115}{324}$	
BM.	P_1:	long-yellow ♀ × rudimentary-gray ♂			
	F_1:	long-gray ♀ × long-yellow ♂			
F_2:	♀ ♀,	l.g. 591,	l.y. 549		
	♂ ♂,	l.g. 228,	l.y. 371,	r.g. 20,	r.y. 3
	P_1:	long-gray ♀ × rudimentary-yellow ♂			
	F_1:	long-gray ♀ × long-gray ♂			
F_2:	♀ ♀,	l.g. 152			
	♂ ♂,	l.g. 42,	l.y. 29,	r.g. 0,	r.y. 0
		Proportion of crossovers,		$\frac{260}{693}$	
COP.	P_1:	vermilion ♀ × white ♂			
	F_1:	red ♀ × vermilion ♂			
F_2:	♀ ♀,	r. 320,	v. 294		
	♂ ♂,	r. 86,	v. 206,	w. 211	
		(7 of the vermilion ♀♀ known from tests to be CC, 2 known to be Cc. 7 white ♂ ♂, Pp, 2 pp.)			
	Back cross. F_1 red ♀♀ from above × white ♂ ♂ gave				
F_2:	♀ ♀,	r. 195,	w. 227,		
	♂ ♂,	r. 66,	v. 164,	w. 184	

Table 8 *(continued)*

		Out cross, F_1 ♀♀ as above × white ♂♂ recessive in P, gave				
	F_2:	♀ ♀,	r. 35,	v. 65,	w. 98	
		♂ ♂,	r. 33,	v. 75,	w. 95	
			Proportion of crossovers,		$\frac{224}{748}$	
COR.		P_1:	miniature-white ♀ × long-red ♂			
		F_1:	long-red ♀ × miniature-white ♂			
	F_2:	♀ ♀,	l.r. 193,	l.w. 109,	m.r. 124,	m.w. 208
		♂ ♂,	l.r. 202,	l.w. 114,	m.r. 123,	m.w. 174
		P_1:	long-white ♀ × miniature-red ♂			
		F_1:	long-red ♀ × long-white ♂			
	F_2:	♀ ♀,	l.r. 194,	l.w. 160		
		♂ ♂,	l.r. 52,	l.w. 124,	m.r. 97,	m.w. 41
			Proportion of crossovers,		$\frac{563}{1561}$	
			or, adding such available figures from Morgan (1911d) and Morgan and Cattell (1912) as are not complicated by the presence of yellow or brown flies,			
					$\frac{1643}{4749}$	
COM.		P_1:	long-white ♀ × rudimentary-red ♂			
		F_1:	long-red ♀ × long-white ♂			
	F_2:	♀ ♀,	l.r. 157,	l.w. 127		
		♂ ♂,	l.r. 74,	l.w. 8 2,	ru.r. 3,	ru.w. 2
			Proportion of crossovers,		$\frac{76}{161}$	
OP.		P_1:	black-red ♀ × black eosin-vermilion ♂			
		F_1:	black-red ♀ × black-red ♂			
	F_2: (all black)		♀ ♀,	r. 885		
			♂ ♂,	r. 321,	v. 125,	c. 122,
			e.-v. 268			
			Proportion of crossovers,		$\frac{247}{836}$	
OR.		P_1:	long-red ♀ × miniature-eosin ♂			
		F_1:	long-red ♀ × long-red ♂			
	F_2:	♀ ♀,	l.r. 408			
		♂ ♂,	l.r. 145,	l.e. 67,	m.r. 70,	m.e. 100

Table 8 *(continued)*

	P_1:	long-eosin ♀ × miniature-red ♂			
	F_1:	long-red ♀ × long-eosin ♂			
F_2:	♀ ♀,	l.r. 100,	l.e. 95		
	♂ ♂,	l.r. 27,	l.e. 54,	m.r. 56,	m.e. 19
		Proportion of crossovers,		$\frac{183}{538}$	
OM.	P_1:	long-eosin ♀ × rudimentary-red ♂			
	F_1:	long-red ♀ × long-eosin ♂			
F_2:	♀ ♀,	l.r. 368,	l.e. 266		
	♂ ♂,	l.r. 194,	l.e. 146,	ru.r. 40,	ru.e. 24
		Proportion of crossovers,		$\frac{218}{404}$	
CR.	P_1:	long-white ♀ × miniature-eosin ♂			
	F_1:	long-eosin ♀ × long-white ♂			
F_2:	♀ ♀,	l.e. 185,	l.w. 205		
	♂ ♂,	l.e. 54,	l.w. 147,	m.e. 149,	m.w. 42
	P_1:	long-eosin ♀ × miniature-white ♂			
	F_1:	long-eosin ♀ × long-eosin ♂			
F_2:	♀ ♀,	l.e. 527			
	♂ ♂,	l.e. 169,	l.w. 85,	m.e. 55,	m.w. 128
		Proportion of crossovers,		$\frac{236}{829}$	
CM.	P_1:	long-white ♀ × rudimentary-eosin ♂			
	F_1:	long-eosin ♀ × long-white ♂			
F_2:	♀ ♀,	l.e. 328,	l.w. 371		
	♂ ♂,	l.e. 112,	l.w. 217,	ru.e. 4,	ru.w. 0
		Proportion of crossovers,		$\frac{112}{333}$	
PR.	P_1:	long-vermilion (yellow) ♀ × miniature-red (yellow) ♂			
	F_1:	long-red-yellow ♀ × long-vermilion-yellow ♂			
F_2: (all y.)		♀ ♀,	l.r. 138,	l.v. 110	
		♂ ♂,	l.r. 8,	l.v. 117,	m.r. 97,
			m.v. 1		
	P_1:	long-vermilion (gray) ♀ × miniature-red ♂			
	F_1:	long-red ♀ × long-vermilion ♂			
F_2:	♀ ♀,	l.r. 116,	l.v. 110		
	♂ ♂,	1.r. 2,	1.v. 81,	m.r. 96,	m.v. 1

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Table 8 *(continued)*

	P$_1$:	miniature-red ♀ × long-vermilion ♂			
	F$_1$:	long-red ♀ × miniature-red ♂			
F$_2$:	♀ ♀,	l.r. 45, m.r. 49			
	♂ ♂,	l.r. 1, l.v. 27, m.r. 26, m.v. 0			
	F$_1$:	long-red ♀ ♀, from above × miniature-red ♂ ♂ of other stock, gave			
F$_2$:	♀ ♀,	l.r. 74,	m.r. 52		
	♂ ♂,	l.r. 3,	1.v. 66,	m.r. 46,	m.v. 1
		Proportion of crossovers,		$\frac{17}{573}$	
PM.	P$_1$:	long-vermilion ♀ × rudimentary-red ♂			
	F$_1$:	long-red ♀ × long-vermilion ♂			
F$_2$:	♀ ♀,	l.r. 451,	l.v. 417		
	♂ ♂,	l.r. 105,	l.v. 316,	ru.r. 33,	ru.v. 4
		Proportion of crossovers,		$\frac{109}{405}$	
OPR.	P$_1$:	long-vermilion ♀ × miniature eosin ♂			
	F$_1$:	long-red ♀ × long-vermilion ♂			
F$_2$:	♀ ♀,	l.r. 205,	1.v. 182		
	♂ ♂,	l.r. 1,	l.v. 109,	l.e. 8,	l.e.-v. 53,
		m.r. 49,	m.v. 3,	m.e. 85,	m.e.-v. 0
BOM.	P$_1$:	long-red-yellow ♀ × rudimentary-eosin-gray ♂			
	F$_1$:	long-red-gray ♀ × long-red-yellow ♂			
F$_2$:	♀ ♀,	l.r.g. 530,	l.r.y. 453		
	♂ ♂,	l.r.g. 1,	l.r.y. 274,	l.e.g. 156,	l.e.y. 0,
		ru.r.g. 0,	ru.r.y. 4,	ru.e.g. 4,	ru.e.y. 0
BOPR.	P$_1$:	long-vermilion-brown ♀ × miniature-eosin-black ♂			
	F$_1$:	long-red-black ♀ × long-vermilion-brown ♂			
F$_2$:	♀ ♀,	l.r.bl. 305,	l.r.br. 113,	l.v.bl. 162,	l.v.br. 256
	♂ ♂,	l.r.bl. 0,	l.r.br. 2,	l.v.bl. 3,	l.v.br. 185,
		l.e.bl. 9,	l.e.br. 0,	l.e.-v.bl. 127,	l.e.-v.br. 0,
		m.r.bl. 1,	m.r.br. 76,	m.v.bl. 1,	m.v.br. 10,
		m.e.bl. 208,	m.e.br. 3,	m.e.-v.bl. 0,	m.e.-v.br. 0

POSSIBLE OBJECTIONS TO THESE RESULTS

It will be noted that there appears to be some variation in coupling strength. Thus, I found (CO) R to be 36.7; Morgan and Cattell obtained the result 33.9; for OR I got 34.0, and for CR, 28.5. The standard error for the difference between (CO) R (all figures) and CR is 1.84 percent, which means that a difference of 5.5 percent is probably significant (Yule 1911, p. 264). The observed difference is 6.1 percent, showing that there is some complication present. Similarly, BM gave 37.6, while OM gave 54.0 — and BOM gave 36.7 for BM, and 36.5 for OM. There is obviously some complication in these cases, but I am inclined to think that the disturbing factor discussed below (viability) will explain this. However, experiments are now under way to test the effect of certain external conditions on coupling strength. It will be seen that on the whole when large numbers are obtained in different experiments and are averaged, a fairly consistent scheme results. Final judgment on this matter must, however, be withheld until the subject can be followed up by further experiments.

Another point which should be considered in this connection is the effect of differences in viability. In the case of P and M, used above as an illustration, the rudimentary-winged flies are much less likely to develop than are the longs. Now if the viability of red and vermilion is different, then the longs do not give a fair measure of the linkage, and the rudimentaries, being present in such small numbers, do not even up the matter. It is probable that there is no serious error due to this cause except in the case of rudimentary crosses, since the two sides will tend to even up, unless one is very much less viable than the other, and this is true only in the case of rudimentary. It is worth noting that the only serious disagreements between observation and calculation occur in the case of rudimentary crosses (BM, and (CO) M). Certain data of Morgan’s now in print, and further work already planned, will probably throw considerable light on the question of the position and behavior of this factor M.

SUMMARY

It has been found possible to arrange six sex-linked factors in Drosophila in a linear series, using the number of crossovers per 100 cases as an index of the distance between any two factors. This scheme gives consistent results, in the main.

A source of error in predicting the strength of association between untried factors is found in double crossing over. The occurrence of this phenomenon is demonstrated, and it is shown not to occur as often as would be expected from a purely mathematical point of view, but the conditions governing its frequency are as yet not worked out.

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These results are explained on the basis of Morgan's application of Janssens' chiasmatype hypothesis to associative inheritance. They form a new argument in favor of the chromosome view of inheritance, since they strongly indicate that the factors investigated are arranged in a linear series, at least mathematically.

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