THE MECHANISM OF CROSSING-OVER III

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V. AN EXPERIMENT TO DETERMINE THE LINKAGE OF MANY FACTORS SIMULTANEOUSLY

A MORE exact knowledge of the interference of one crossing-over with another required an experiment, or series of experiments, in which the distance between the two points of crossing-over in cases of double crossing-over could be more accurately determined. In an experiment involving only three factors—A, D and H—if a double cross-over occurs, all that can be known is that crossing-over has occurred at the same time somewhere between A and D, and somewhere between D and H, but nothing can be known of the precise location and distance apart of the two points of crossing-over, except that they could not be further apart than A and H. On the other hand, if the inheritance of four points could be followed—say, A, D, F and H—then the distance between the two points of crossing-over could be determined a little more exactly, for a double crossing-over involving breaks between A and D, and between D and F, would cut out a shorter segment of the chromosome than one occurring in regions A–D and F–H. And the more numerous were the factors that could be followed—other things being equal—the more exact the determination would become. At the same time, it might be possible by comparing the results of a *series* of different experiments to reach the end desired by the two or three-factor method also. For example, the difference in frequency between the double cross-overs obtained in an experiment involving A, B, C and in an experiment involving A, B, D, must obviously be due to the double cross-overs involving regions A–B and C–D,[1] except in so far as these differences are due to the random deviation of different samples from each

[1] For region BD is made up of BC + CD. Therefore double crossovers involving AB and BD really consist of double cross-overs involving AB and BC plus those involving AB and CD. Consequently, if we subtract the number of double cross-overs involving AB and BC from the number involving AB and BD, we obtain the number involving AB and CD.

other, or to actual differences in the behavior of the chromosomes in the two experiments. As the last two influences seemed by no means negligible, and as the experiment involving many points at once gave a more direct and graphic picture of the results, it was decided to use this method of attack in preference. Moreover such an experiment incidentally afforded an opportunity of attacking certain other questions, such as the effect, on crossing-over, of having the two chromosomes different in regard to many factors. Meanwhile, the indirect method of attack would be followed by various workers, and the two sets of results could finally be used as checks upon each other.

The many factor method is in itself for several reasons very laborious, but this is compensated for by the fact that when the results are obtained they are the equivalent of an entire series of different experiments involving in turn the linkage of each factor with every other one, and indeed, the results are much more than the equivalent of these, for in the latter cases the linkages are obtained in different experiments, so that there is much more chance for error in determining the relation of one linkage to another.

It was evident from the outset, however, that there was one very important obstacle to be overcome in any study of linkage exact enough to give useful information regarding coincidence, and that the difficulty was especially great in the type of experiment contemplated. The difficulty referred to is "differential viability," for it is found that in nearly all experiments not involving the characteristics of seeds or other structures dependent upon the maternal organism for support, the individuals belonging to different genetic classes may be very differently equipped in respect to their ability to meet the struggle for existence. Thus, since the count generally takes note only of the individuals which survive, the ratios obtained may be very different from the ratios of the different classes of gametes. These discrepancies apply especially to forms like flies, the larval life of which can not be well controlled, and they are, of course, particularly great in crosses involving many factors at once.

Before considering the means by which differential viability may be reduced in crosses of multiple stocks, it may not be out of place to explain two methods I devised for getting a more correct estimate of the gametic ratio in back-crosses involving only two pairs of linked factors,

Let us say that the gametic ratio is r(AB):r(ab): s(Ab):s(aB). Assume that when A is present the viability of the flies is reduced so that only A′ per cent. of those which would otherwise survive, now come to maturity, and assume that factor B lessens the output to B′ per cent. of what it otherwise would be; similarly a and b, when present, lower the output to a′ per cent. and b′ per cent., respectively. Then the relative number of AB individuals which survive will be rA′B′ (per cent. marks are omitted for brevity); the relative number of Ab will be sA′b′, etc. The actual, observed, numbers will be some multiple (k) of these relative numbers; thus the number of AB individuals actually found will be krA′B′, the actual number of Ab will be ksA′b′, etc. It can now be shown that the true gametic ratio (r:s), which it was desired to find, may be derived by the formula

$$\sqrt{\frac{AB \times ab}{Ab \times aB}}$$

(using Ab, ab, etc., to denote the number of AB observed, of ab observed, etc.), for, substituting the above values of AB, ab, etc., in this formula, we obtain

$$\sqrt{\frac{krA'B' \times kra'b'}{ksA'b' \times ksa'B'}} = \sqrt{\frac{k^2r^2A'B'a'b'}{k^2s^2A'b'a'B'}} = \sqrt{\frac{r^2}{s^2}} = \frac{r}{s}.$$

This formula should be used only when the smallest class has not a very large probable error, for, by multiplying the value of this class in the formula, we give the entire result a probable error proportional to that of the smallest class. Another objection to the formula is that it assumes that each factor produces the same specific lowering of viability, independently of whatever other factor it comes into combination with; this is not always true, since factors often produce different effects when in different combinations.

The two above difficulties are avoided by the second method, the main feature of which consists in making two different kinds of crosses in preparation for the linkage determination: *i. e.*, cross AB by ab, and what I have termed the "*contrary cross*," Ab by aB. A back-cross of the F_1 from the first cross gives the gametic ratio r (AB) : r (ab) : s (Ab) : s (aB); and the other cross results in gametes showing the proportion s (AB) : s (ab) : r (Ab) : r (aB). Suppose that w per cent. of AB individuals are viable, x per cent. of ab, y per cent. of Ab, and z per cent. of aB. Then in the first cross the observed ratio would be r w (AB) : r x (ab) : s y (Ab) : s z (aB), and, in the second cross, s w (AB) : s x (ab) : r y (Ab) : r z (aB). The numbers actually observed in the crosses would be some multiple of these ratios, but a different multiple in the two cases. Thus we could designate the numbers actually observed in the first cross as krw(AB) : krx(ab) : ksy(Ab) : ksz(aB), and the numbers in the second cross as csw(AB) : csx(ab) : cry(Ab) : crz(aB).

In this case the ratio r : s may be obtained by the following formula:

$$\sqrt{\frac{AB_1 \times Ab_2}{AB_2 \times Ab_1}}$$

(using the symbol AB_1 to denote number of AB observed in the first cross, Ab_2 to denote number of Ab observed in the second cross, etc.). Now, the value of AB_1 has already been given as krw, of Ab_2 as cry, etc. Substituting these values in the above formula, we obtain

$$\sqrt{\frac{krw \times cry}{csw \times ksy}} = \sqrt{\frac{r^2kcwy}{s^2ckwy}} = \sqrt{\frac{r^2}{s^2}} = \frac{r}{s}.$$

Besides this formula involving AB and Ab, there are three similar formulas which will also give the gametic ratio, namely:

$$\frac{AB_1 \times aB_2}{AB_2 \times aB_1}; \qquad \sqrt{\frac{ab_1 \times Ab_2}{ab_2 \times Ab_1}}; \qquad \sqrt{\frac{ab_1 \times aB_2}{ab_2 \times aB_1}}.$$

That formula should usually be chosen which contains the largest number of individuals in its smallest class, for this would usually have the least probable error.

This method makes no assumption as to an independent action of the different factors in reducing viability. It does assume, however, that for individuals with the same combination of factors there is the same degree of viability in the two experiments; this is not always true, since under different conditions of food, etc., individuals of the same genetic type may have very different degrees of viability; moreover, there are sometimes "invisible" factors present in one experiment but not in the other which influence viability and which are linked with the factors that are being studied. The assumption, nevertheless, is unavoidable. But it can be shown mathematically that any errors in the calculated values, due to assumptions made in following the formulas of either the first or the second method, are greatly reduced by using a combination of the two methods; namely, by making "contrary crosses," calculating the linkage value in each of them by means of the first method, and then taking the square root of the product of these two values.

In a cross involving three or more factors no formula corresponding to the one first given is possible, and before it is possible to use a formula corresponding to the second method, an increasingly large number of different kinds of crosses must be made, according to the number of factors involved. Still another method is, therefore, necessary in order to obtain fairly accurate results from crosses involving many factors, except in the rare case that these factors have very little differential effect on viability. The method devised is as follows:

The female, heterozygous for many factors, whose gametic output it is desired to study, is back-crossed, not to a multiple recessive male, but to one homozygous for all, or nearly all, the *dominant* factors (these are, in the case of flies, mostly the normal allelomorphs). All the offspring appear alike, then, in that they all show the dominant characters of their father (except in the case of sex-linked factors, which are transmitted by the father to his daughters only), and so all should be of the same viability, except for the insignificant effect of the recessive factors

present in heterozygous condition (and the effect of the one or two characters wherein the father may not have been dominant). Thus error due to differential viability may be held within safe bounds.

It may be objected, however, that we have, as it were, killed the patient in curing the disease—that there is no use in overcoming the discrepancies in the count due to differential viability, if we thereby eliminate the possibility of making any count at all, by making all the offspring appear alike! It is true that, in such an experiment, it is impossible to tell *by inspection* of any offspring, what maternal factors were present in the ova from which they sprang, since these factors are made invisible, so to speak, by the dominant factors brought in by the sperm. But the factorial composition of each of these offspring (which we will for convenience call "F_2") can be determined by breeding tests. The plan which was followed was to mate the F_2 flies, each in a separate bottle, to individuals containing the recessive factors. Thus whatever recessive factors were present in the eggs of the original heterozygous female ("F_1"), whose output it was desired to test, would become visible the generation after (in "F_3"). Whereas, in an ordinary linkage determination, each bottle produces a large number of flies, which need merely be classified according to their appearance, and counted—in this case, each of the offspring themselves requires to be mated and given a whole bottle to itself, and its progeny in turn ("F_3") must be examined. In other words, in ordinary cases, there is only one bottle necessary for a count of many flies, but in this case one bottle represents one fly of the count. The numerical relations existing between the flies ("F_3") hatching in one of these final testing-out bottles need not be determined, however; that is, these flies need not be counted; all that is necessary is a "qualitative" determination of what recessive characters appear among them, in order to judge of the composition of their parent (F_2), which is the fly recorded in the count. In all, 1008 of these test bottles have been recorded.

In preparation for this experiment the main task was to

secure stock that contained many mutant, linked factors at the same time. But, as was explained in the account of experiments with the third chromosome, it is necessary, in dealing with linked factors, to make the crosses in a particular way to secure a "multiple stock." Thus, it may be pointed out again here, a stock containing factors A, B, C cannot be obtained ordinarily by crossing stock A to stock C, and then crossing the double stock A C (produced in F_2, F_3, or F_4 from the first cross) to stock B; because it would require double crossing-over for the hybrid fly, containing A and C in one chromosome, and B in the other, to produce a gamete with A, B and C all in the same chromosome (assuming the factors to be linked in this order). If the linkage is tight such double crossing-over will never occur. But by first obtaining stock A B and then crossing this to C, stock A B C may be secured; for in the hybrid fly that contains A B in one chromosome and C in the other, a crossing-over between B and C will result in a chromosome that contains A, B and C, the link between A and B not having been broken.

In other words, the factors can only be added together in a certain order, owing to their position in the linkage chain. Just as in adding links to a chain, one or more factors cannot be wedged in *between* factors in another collection (except by double crossing-over); but if they lie *beyond* this collection, they may be added on, either singly or in a group. The information that had already been gained by Sturtevant, Morgan and Bridges concerning the order in which various factors lay, was therefore of great service in determining how the crosses should be made, to get the factors together, and besides this several double stocks of a sort that could be used in the present experiment had already been synthesized by them. But the progress of the experiment was very considerably retarded by the fact that the position of a number of the factors which it was desired to use had not yet been determined. These comprised bifid and forked in chromosome I and dachs, jaunty, curved, arc and balloon in II. (The exact position of jaunty with respect to black, and of

balloon with respect to speck is still unknown.) Various "trial and error" matings were therefore made in the hope of getting these unplaced factors in suitable combinations, and crosses were also undertaken to secure such data in regard to their position as would be useful for the purpose in view. These attempts were often cut short, owing to the information which was meanwhile being accumulated by the other workers, but before the latter information was obtained the positions of bifid, forked and dachs had been determined, and several multiple stocks that were later used had been made up.

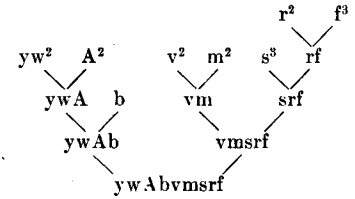
We may now consider specifically what combinations of factors were actually employed in the experiment and what special methods were used for securing and maintaining these combinations.

In the case of the first chromosome, it was desired, for the final linkage determination, that the heterozygous flies, whose gametic output was to be tested, should contain most of the recessive factors (which are usually the mutant ones) in one chromosome, and the dominant (usually normal) factors in the other, for it was considered worth while to test the possibility that a chromosome containing so many mutant factors might behave abnormally. Furthermore, if the commonly accepted belief were correct, that recessive factors are "absences," it was possible that the chromosome with many recessive factors might be shorter than the other, and that linkage disturbances might arise for this reason. Two stocks were, therefore, made up, of the factors in the first chromosome, to supply these two kinds of chromosomes for the heterozygous females to be tested. One stock contained the mutant factors for yellow body color, white eyes, abnormal abdomen, bifid wings, vermilion eyes, miniature wings, sable body color, rudimentary wings, and forked spines. All of these factors are recessive except abnormal abdomen, which is only partially and irregularly dominant. The other stock contained only the mutant factors cherry eye, club wing, and bar eye. Cherry is an allelomorph to the factor for white eyes carried by the other chromosome,

and is dominant to it, though not completely; club is recessive; bar is dominant (somewhat incompletely).

The reason that club was not put into the series with the other recessives is that it was discovered (by Bridges) after this series had already been put together, and so it would have required taking the stock apart again, or else obtaining a rare double cross-over, to wedge club into this series. It was a valuable factor to have in the experiment, however, since it lay in a region of the chromosome where there were no other mutant factors to give data as to crossing-over. Accordingly, it was inserted in the other series. It will be observed, however, that, in spite of this, one chromosome contains 7 more dominants than the other.

The order of the above factors is y, w or c, A, b, c_1, v, m, s, r, f, B_r. In making up the first stock the factors were put together as follows (omitting from consideration all trial or discarded combinations):



Of course the putting together of factors from two stocks, although shown above as only one step in each case, always requires several generations. Moreover, as will be seen below, these steps do not usually consist in getting the ordinary F_2 or back-cross, in the case of the complicated combinations. This is partly because of the serious obstacle which the poor viability of flies having many mutant characters presents to the making up of multiple stock, just as it does to the securing of counts from it; moreover, in the making up of stock, the sterility of such flies is an equally important difficulty.

These difficulties were overcome here in much the same way as they were in making the counts—namely, by keeping the stock, so far as possible, heterozygous. For ex-

[2] From Morgan.

[3] From Bridges.

ample, in the last step shown in the preceding diagram, where factors ywAb and vmsrf are to be put together, it was found that females of both of these kinds were extremely difficult to keep alive. It was, therefore, decided to mate a vmsrf male by a female which contained ywAb in one chromosome and normal factors in the other. Such a female would be easy to breed from, as the normal factors dominate. About half the daughters (let us call them F_1) would be of composition $\frac{ywAb}{vmsrf}$ (representing the mutant factors in the maternally derived chromosome on the upper line, those from the father on the lower line). All the daughters (F_1) would, however, appear normal, but if these F_1 females were bred in separate bottles, those of the desired composition $\frac{ywAb}{vmsrf}$ would be distinguishable from the others by their offspring (F_2). All bottles in which the parents (F_1) had not been of the desired composition could then be discarded. Next, among the offspring (F_2) of those females which proved to be of composition $\frac{ywAb}{vmsrf}$, it was necessary to select the ones which, by reason of crossing-over between b and v, contained all nine factors in the same chromosome (*i. e.*, ywAbvmsrf). But such individuals, if homozygous, never live long enough to mate, so great is the lowering of viability produced by all these mutant factors at once. Consequently, some method must be used of obtaining in this cross heterozygous individuals (F_2) which received this cross-over "nontuple" chromosome from their mother, and of distinguishing these from other individuals produced by the cross. The natural suggestion would then be that the F_1 females should be mated by normal males, and the F_2 which receive this cross-over chromosome could then be distinguished by breeding tests as their mother had been. The crossing-over desired, however, does not occur in more than one eighth of the flies, and so breeding tests designed to be certain of securing at least one individual of the required composition would have to be rather extensive. In this case, however, the desired F_2

flies can be "spotted" in another way, without breeding tests, and yet without making them homozygous for many mutant factors and thus inviable. The method used was to mate the F_1 females to bv males, which had been made up for this special purpose. The b v daughters (F_2) must be cross-overs, since in the F_1 mother b and v were in different chromosomes; moreover, a glance at the formula of the F_1 females will show that these cross-over chromosomes must have been formed of the left-hand end of the upper chromosome and the right-hand end of the lower. Thus these bv females contain a chromosome with all nine mutant factors (except in the case of the few double cross-overs). Since, however, they were homozygous in only two mutant factors, they could easily be bred.

A similar scheme was used in many of the other steps shown in the diagram representing combinations made in group I, and was also used frequently in group II. Owing to the fact that rudimentary winged females (group I) are practically sterile, devices of this sort had to be used in dealing with flies containing this factor from the very start, and the same may be said of flies with dachs legs (group II), since these also were found very hard to handle. In most of the other cases, however, it was not necessary to use such a method before several factors had been combined together, as flies homozygous for just two or three mutant factors were generally viable enough to handle. There would be no object in wearying the reader with a description of the exact way in which each of the steps was taken; it is the author's purpose only to explain the nature of methods used, giving only sufficient examples to make clear the details of any devices never previously employed that might be capable of application to other cases.

From the example of the cross involving bv, previously given, we may now generalize, and establish the rule that in making up, and also in keeping stocks containing many linked recessive factors, if the latter cause a marked lessening of fertility or viability, it is best to follow the practise of keeping the stocks heterozygous, by back-crossing them to stocks containing only the few recessive (or par-

tially recessive) factors necessary to show which offspring contain the desired cross-over or non-cross-over chromosome. In the example, this method was used in combining two stocks to make up a recombination stock. The same means is employed in maintaining the multiple stock after it has been synthesized. Thus, in the case of group I, the females containing in one chromosome the combination ywAbvmsrf (the "F_2" obtained above), were crossed to cc_1B_r males. In this way some daughters (F_3) are produced (which these were was determined by breeding tests) that received from their mother ywAbvmsrf, and from their father cc_1B_r. These F_3 females having the composition $\frac{\text{ywAbvmsrf}}{c \quad c_1 \quad B_r}$, were then back-crossed to cc_1B_r males again, in order to maintain the stock. Since all the daughters (F_4) received cc_1B_r from their father, those which do not show these characters fully developed must have received from their mother factors near both ends of the chromosome containing the nine mutant factors. Therefore, except for the very few flies in which crossing-over occurred between w and y, which is at the very end, or in which double crossing-over occurred, all the light cherry, normal winged, partially bar eyed flies will have a composition like that of their mother, and may be bred in the same way, again to the cc_1B_r males, which now hatch from the same bottle. This then is a cross exactly like the preceding one, except for the few cross-over flies above mentioned. The latter may be detected, however, and their offspring discarded if the females are bred in separate bottles. This same cycle may be repeated generation after generation. Thus a continual supply is maintained of flies heterozygous for all these factors.

In making the linkage determinations, such flies are bred to normal or to bar males, and the female offspring, which are all alike in appearance except in respect to the partially dominant factors A and B_r, and which should, therefore, have had approximately equal chances for surviving, are individually tested for their contained characters. For the tests, the female need not be virgin, since, whatever kind of male is employed, the sons will show only

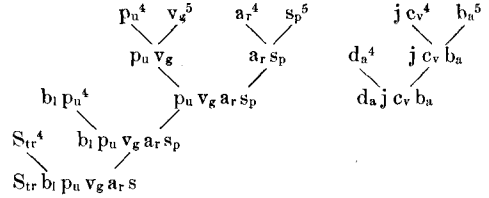
those sex-linked characters that their mother contained and they may therefore be used to determine the composition of their mother. As a matter of fact, however, males containing v were generally employed, so that v, if it had been present in the tested female, would appear in her daughters as well as her sons. This additional test for v was desirable because it is a factor which in a white, cherry, or bar eye it is difficult or impossible to detect.

Stock of the second chromosome was obtained, and is maintained, in an essentially similar way. Here the attempt was not made to put most of the mutant factors into one of the two chromosomes of the heterozygous females to be tested. This was partly because an experiment of this sort with one chromosome would seem sufficient. Moreover, it was harder to make up multiple stocks of the second chromosome, since the order of fewer factors had at this time been well determined, and since, besides, it takes a greater number of generations to put non-sex-linked factors together into the same stock than it does to put sex-linked factors together. For, if two recessive stocks of chromosomes II are crossed, the F_1 males, in which crossing-over never occurs, transmit the recessive factors of only one stock to each son and daughter. The latter then can not be homozygous for both sets of recessive factors, and so it is impossible to pick out, except by further breeding, those that received both sets from the mother. But as in the case of the bv illustration given, if a male with C D is available to cross with the "F_1" hybrid female $\frac{ABC}{DEF}$, the "F_2" individuals showing both characters C and D must have the composition $\frac{ABCDEF}{CD}$, and so the desired cross-overs may be picked out immediately in F_2.

A somewhat similar scheme, often especially useful for obtaining desired combinations in a non-sex-linked group, involved making use of cross-overs that break combinations already obtained. This too may be shown by an example. It was desired to obtain a stock containing the second chromosome factors dachs legs, jaunty wings, curved wings, and balloon wings. Dachs black stock al-

ready had been made up, as had j c_v b_a. These two stocks were crossed together, and the F_1 was back-crossed to d_a b_1. In the F_2 generation all the dachs that are *not* black are cross-overs in the region between these two factors, and so must contain, in the same chromosome with dachs, instead of black, the factors j c_v and b_a, for black is in such a position in the chromosome that a cross-over between d_a and b_1 must nearly always be a cross-over between d_a and j c_v b_a.

In the case of the second chromosome the individuals tested for linkage contained, in one chromosome, the factors streaked thorax, black body color, purple eyes, vestigial wings, arc wings, and specked thorax, and in the other chromosome, the factors dachs, jaunty, curved and balloon. The order of these factors is as follows: S_{tr} d_a b_1 p_u v_g c_v a_r s_p b_a. The way in which they were combined is as follows:



As d_a j c_v b_a is very inviable it is kept in heterozygous condition by back-crossing, in each generation, normal appearing males of the composition $\frac{d_a\ j\ c_v\ b_a}{b_1\ p_u\ v_g\ a_r\ s_p}$ (called ⅘ for short) to b_1 p_u v_g a_r s_p females (called 5). Since there is no crossing-over in the male, all the offspring are either apparently normal, ⅘, or the homozygous quintuple recessive, "5." The same process can then be repeated in every generation, by crossing the normal-appearing sons to their recessive sisters. It is evident that the "5" females which are used need not be virgin, as they could have been fertilized only by ⅘ or by 5 males. When ⅘ males are crossed by 5 females which have been made up so as to contain in addition the dominant factor streak (these 5 females need only be heterozygous for streak; homozygous streaks are hard to handle), the daughters

[4] From Bridges.

[5] From Morgan.

which appear streaked but otherwise normal, must have the composition: $\frac{d_a\ j \quad c_v \quad b_a}{S_{tr}\ b_1\ p_u\ v_g\ a_r\ s_p}$. These are the "$F_1$" females whose gametic output is to be tested. Accordingly, they are crossed to normal males. All the offspring ("F_2") appear normal (except for the dominant, streak), but the factors they received from their mother may be determined by mating them, individually, to ♂♂ males, for the latter contain (heterozygous) all recessive characters possible in the former.

It was at first thought that labor might be saved, and certain points in addition determined, by conducting the linkage determinations on flies heterozygous for the factors used in both chromosomes I and II at the same time, instead of making determinations of the linkage in the two chromosomes in separate experiments. The multiple stocks of the two chromosomes were, therefore, crossed together, and females were finally obtained that had the composition:

$$\frac{y\ w\ A\ b\ v\ m\ s\ r\ f}{c \quad c_1 \qquad B_r} \qquad \frac{S_{tr}\ b_1\ p_u\ v_g\ a_r\ s_p}{d_a\ j \quad c_v \quad b_a}$$

These females, heterozygous for 22 mutant factors, were then crossed to normal males, and the composition of their female offspring was tested by mating these in separate bottles to ♂♂ males. The maintenance of the double-multiple stocks proved to be extremely difficult, however, and so, after obtaining determinations for 166 offspring from such females, the two groups of mutant factors were again separated. The data obtained in this part of the experiment show that there is no linkage of any of the twelve factors studied in group I with any of the ten studied in group II; this is of course in marked contrast to the relations shown between factors in the same group. The conclusions of previous workers that no factor in one group was linked with any factor in another group were based on results obtained with comparatively few combinations of factors, which were chosen as samples, so to speak. It will be seen that in the present work these conclusions have been confirmed by a study of 132 differ-

ent combinations of factors in group I with factors in group II.

CLASSIFICATION OF FACTOR COMBINATIONS TRANSMITTED BY FEMALES HAVING THE COMPOSITION: $\frac{y\ w\ A\ b\ v\ m\ s\ r\ f}{c \quad c_1 \quad B_r}$

	Yellows		Grays		Totals
Non-cross-overs					
	ywAbvmsrf	186	*c c₁ B_r*	200	386
Between	*Single Cross-overs*				
y and w	*yc c₁ B_r*	2	*wAbvmsrf*	5	7
w and A	*yw c₁ B_r*	3	*cAbvmsrf*	5	8
A and b	*ywA c₁ B_r*	4	*c bvmsrf*	11	15
b and c₁	*ywAb c₁ B_r*	17	*c vmsrf*	27	44
c₁ and v	*ywAb B_r*	46	*c c₁ vmsrf*	51	97
v and m	*ywAbv B_r*	7	*c c₁ msrf*	9	16
m and s	*ywAbvm B_r*	18	*c c₁ srf*	19	37
s and r	*ywAbvms B_r*	28	*c c₁ rf*	38	66
r and f	*ywAbvmsr B_r*	0	*c c₁ f*	5	5
f and B_r	*ywAbvmsrf B_r*	0	*c c₁*	1	1
Between	*Double Cross-overs*				
y and w; c₁ and v	*yc c₁vmsrf*	1	.....		1
y and w; m and s	.....		*wAbvm B_r*	1	1
y and w; s and r	*yc c₁ rf*	1	*wAbvms B_r*	1	2
y and w; r and f	*yc c₁ f*	1	.....		1
w and A; c₁ and v	*yw c₁vmsrf*	1	.....		1
w and A; r and f	*yw c₁ f*	1	.....		1
A and b; c₁ and v	.....		*c b B_r*	1	1
A and b; s and r	*ywA c₁ rf*	1	.....		1
b and c₁; m and s	*ywAbc₁ srf*	1	*c vm B_r*	1	2
b and c₁; s and r	*ywAbc₁ rf*	4	*c vms B_r*	3	7
c₁ and v; v and m	.....		*c c₁ v B_r*	1	1
c₁ and v; s and r	*ywAb rf*	7	*c c₁ vms B_r*	1	8
c₁ and v; r and f	*ywAb f*	2	.....		2
c₁ and v; f and B_r	*ywAb*	1	.....		1

Total Double and Single Crossing-over

Between	Observed Number	Per Cent. of Crossing-over	Between	Observed Number	Per Cent. of Crossing-over
y and w	12	2	v and m	17	2
w and A	10	1.5	m and s	40	6
A and b	17	2	s and r	84	11.5
b and c₁	52	7.5	r and f	9	1.2
c₁ and v	112	16.	f and B_r	2	0.3

Not only the independence of the factors in the two groups was shown by the experiment in which the two groups were followed at once, but also the independence of the crossings-over. In the total of 166 cases, there were 81 in which chromosome I underwent crossing-over (either

single or double), and 101 in which chromosome II crossed over. If it was a matter of pure chance whether or not crossings-over occurred in I and II at the same time, coincident crossing-over should have happened in $\frac{81}{166} \times 101 = 49+$ cases. The actual number of cases in which crossing-over occurred in both chromosomes at once was 52. Thus there is neither interference nor synchronism of these crossings-over, and this result too is strikingly dissimilar to the relations found between two crossings-over in the same chromosome.

Since the results in the two chromosomes were found to be independent in all respects, it is deemed unnecessary to list here all the different combinations which were found of factors in group I with factors in group II, and their observed frequencies. The results for the two chromosomes may more advantageously be separated and added to the other results, obtained when groups I and II were followed in different crosses.

The data for the first chromosome are given in the table on page 365. In all, 712 offspring of females heterozygous for the 12 mutant factors in group I have been tested.

The above results give a direct demonstration of the fact that the factors behave as though they are joined in a chain; when interchange takes place, the factors stick together in sections according to their place in line and are not interchanged singly. The fact is shown so patently as to require no further comment.

Non-crossing-over occurs in this chromosome in 54.4 per cent. of cases, single crossing-over in 41.6 per cent., double crossing-over in 4.2 per cent. No triple crossing-over was obtained in this count, although one, which will be described later, was obtained in the next generation, in one of the "testing out" bottles.