A CRITICISM OF THE HYPOTHESIS OF *LINKAGE* AND *CROSSING OVER.*

By A. H. TROW, D.Sc., F.L.S.

The publication of *The Mechanism of Mendelian Heredity* by Morgan, Sturtevant, Muller, and Bridges, marks a definite stage in the development of the hypothesis of *linkage* and *crossing over*. The authors' faith in this hypothesis has evidently become so strong that they are not unlikely to infect others with their belief, irrespective of any real demonstration of its validity. It seems therefore desirable that the hypothesis should be subjected to independent criticism. Such criticism is really rendered necessary by the fact that although the authors devote much time and space in their book to the elucidation of the simple Mendelian ratios, they give no clear coherent account of their mode of explanation of the more complex and troublesome ratios which students of genetics classify under the headings *coupling*, *repulsion*, *reduplication*, and *crossing over*.

Drosophila ampelophila, the type which these authors have mainly investigated, appears to be an organism admirably suited to genetic analysis. Already more than a hundred factors have been isolated and their relationships studied in some detail. The authors may be congratulated on their choice of such excellent material, and on the skill and industry with which they are exploiting it. One peculiarity of the organism deserves special mention—the x or n number of chromosomes is four, and the set is remarkable in that one of them is spherical in form, the remaining three being rod-like.

In the study of the hundred factors already recognised in this organism, many deviations from the normal gametic and zygotic ratios have been recognised, and it is these which have apparently led the authors (who reject the reduplication hypothesis) to elaborate their alternative hypothesis of linkage and crossing over.

They interpret the normal Mendelian ratios as due to the presence of the factors in different chromosomes. Thus if two factors A and B give the zygotic ratio of $9:3:3:1$, they interpret this by locating A and B in different chromosomes. It is obvious that if these factors *really* are in different chromosomes, there *can be* no linkage and crossing over, in Morgan's sense, so far as they are concerned. It appears, moreover, from their statements, although the actual evidence is not directly produced in sufficient amount to form a proof, that many factors are linked together in such a way that they behave during the segregation period as an indivisible whole. For example, X, Y, Z may represent characters which are generally completely correlated. Tested separately by crossing with the corresponding recessive, x, y, z, we should find that $\left.\begin{matrix} X:x \\ Y:y \\ Z:z \end{matrix}\right\} :: 3:1$, but, and this is the vital point, generally, though not always, $XYZ : xyz :: 3:1$. Such a behaviour is well described as *linkage*, and would be undiscoverable if it were really absolute. Its discovery only becomes possible when the linkage can be broken down. The genesis of the hypothesis of crossing over is no doubt traceable to the fact that the linkage is not absolute, except in rare cases.

Perhaps the most essential element in the crossing over hypothesis is the conception of the factors as being represented by the chromomeres of the chromosomes, as if indeed they were numbered beads on a string, each having a definite locus of its own. The factors are not only restricted to a special chromosome, but to a special position in this. So much is this the case that the authors have represented in the Frontispiece of their book the position of no fewer than thirty-six factors; in one chromosome alone the positions of as many as nineteen are shown.

The authors take their standpoint (confessedly or by implication) on the following grounds:

(1) that the individuality of the chromosomes may be now accepted as fully established,

(2) that rod-like chromosomes actually occur in *Drosophila*,

(3) that in the prophases of meiosis in certain organisms whose cytology has been more fully studied than that of *Drosophila*, the homologous chromosomes may be observed in the positions required by the hypothesis,

(4) that the percentage of cross-overs obviously! depends upon the distance apart of the factors concerned, and therefore, conversely,

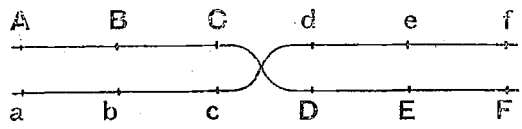
(5) that the percentage of cross-overs enables one to allocate the factors concerned to their proper relative positions.

Let us consider whether the authors' standpoint is a safe one. We may admit the validity of the contention that the chromosomes have an individuality of their own, and that the relations of the chromomeres to each other remain approximately constant—to do otherwise would render all further discussion futile.

We may admit too that in *Drosophila* crossed chromosomes probably occur in meiosis. It should be added that the crossing over hypothesis renders the detailed cytological study of these cross-overs in this organism a matter of pressing importance.

Now, do the homologous chromosomes cross according to the laws of chance, anyhow, anywhere, or according to some geometrical scheme? It is exceedingly difficult to discover whether the authors have really adequately considered this point.

Let us suppose that the cross-over takes place once only, and at the middle point of the chromosome, and let the number of factors (or chromomeres) be reduced to six, and lettered *ABCDEF* and *abcdef*, the chromosome being regarded as a paired chromosome of a nucleus in meiosis of some F_1 plant. We should get the following arrangement:



Crossing over takes place so as to produce the cross-overs

Ad, Ae, Af; *Bd, Be, Bf*; *Cd, Ce, Cf*

aD, aE, aF; *bD, bE, bF*; *cD, cE, cF*

in equal numbers, and the non-cross-overs

AB, AC, BC; *ab, ac, bc*

DE, DF, EF; *de, df, ef*

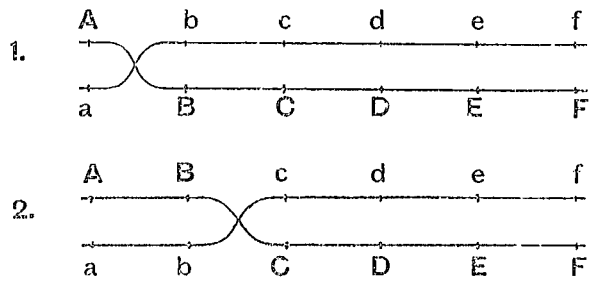
in equal numbers.

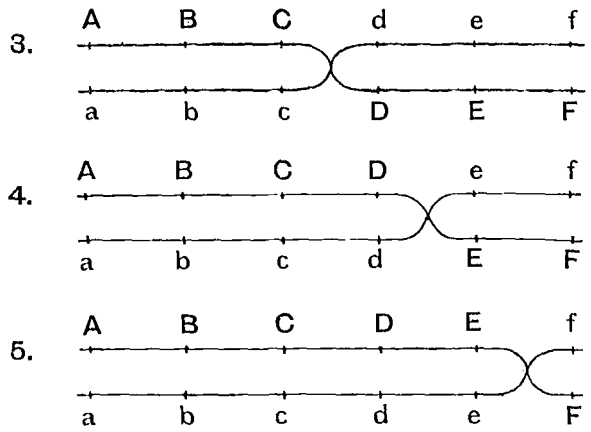
Put in another way, any one of the capital letters *A*, *B*, *C* combines with any one of the small letters *d*, *e*, *f*, and any one of the small letters *a*, *b*, *c* combines with any one of the large letters *D*, *E*, *F* to form *cross-overs*, but there are *no corresponding non-cross-overs*, if *every chromosome has a constant behaviour*. If however for every chromosome which crosses over in this definite way, another similar chromosome in another nucleus does not cross over at all, so that the ♂ and ♀ contributions in this case simply separate from each other, then there would be non-cross-overs present, *AD*, *AE*, *AF*; etc. (along with others) corresponding to the set of cross-overs given above. We should get, if both types occur equally often, equal numbers of cross-overs and non-cross-overs, e.g.,

AD ad	aD Ad
non-cross-overs	cross-overs
50 %	50 %

If two, three, or four chromosomes remained uncrossed for each one that crosses, the percentage of cross-overs would fall to 33 %, 25 %, 20 %, and so on. In such a scheme it is obvious that the percentage of cross-overs does not give any information as to the relative position of the factors in the chromosomes. This behaviour does not depend on the selection of the middle point as the locus of the actual crossing over. If the crossing over takes place at *any one fixed point*, a similar conclusion is inevitable. Hence we may be sure that the authors of the crossing over hypothesis had some other scheme than this in view.

Now if the crossing over does not take place (if it takes place at all) always at the middle part of the chromosome, or some other definite point, let us suppose that it takes place according to the laws of chance at *any one* point. With the same chromosomes as before, with six factors each, there will obviously be five points at which crossing over will take place, as in the following figures:





From these figures we can see that the cross-overs in which A is concerned are as follows:

1.	Ab	Ac	Ad	Ae	Af
2.		Ac	Ad	Ae	Af
3.			Ad	Ae	Af
4.				Ae	Af
5.					Af
In all five cases	$1Ab$	$2Ac$	$3Ad$	$4Ae$	$5Af$
Similarly with B.		$1Bc$	$2Bd$	$3Be$	$4Bf$
C.			$1Cd$	$2Ce$	$3Cf$
D.				$1De$	$2Df$
and E.					$1Ef$

According to this scheme the cross-overs appear to occur in numbers exactly proportional to the distance apart of the factors concerned. For example, F is five units from A, D three units, and B one unit. Af occurs five times, and Ad three times as often as Ab. This is not exactly obvious until it has been worked out, but my mathematical colleagues agree with me that it is perfectly true. This is doubtless the mathematical basis upon which the hypothesis of the localization of the factors has been built up. We have however to note that the essence of the scheme consists in determining the ratio between cross-overs and non-cross-overs. An examination of the figures will show that we get the following relationships:

$\frac{Ab}{AB} = \frac{1 \text{ cross-over}}{4 \text{ non-cross-overs}} = \begin{cases} 20\,\% \text{ of} \\ \text{cross-overs} \end{cases}$ $\quad$ $\frac{Bc}{BC} = \frac{1 \text{ C. O.}}{4 \text{ N. C. O.}} = \begin{cases} 20\,\% \text{ of} \\ \text{cross-overs} \end{cases}$

$\frac{Ac}{AC} = \frac{2 \text{ cross-overs}}{3 \text{ non-cross-overs}} = 40\,\%$ $\quad$ $\frac{Bd}{BD} = \frac{2 \text{ C. O.}}{3 \text{ N. C. O.}} = 40\,\%$

$\frac{Ad}{AD} = \frac{3 \text{ C. O.}}{2 \text{ N. C. O.}} = 60\,\%$ $\quad$ $\frac{Be}{BE} = \frac{3 \text{ C. O.}}{2 \text{ N. C. O.}} = 60\,\%$

$\frac{Ae}{AE} = \frac{4 \text{ C. O.}}{1 \text{ N. C. O.}} = 80\,\%$ $\quad$ $\frac{Bf}{BF} = \frac{4 \text{ C. O.}}{1 \text{ N. C. O.}} = 80\,\%$

$\frac{Af}{AF} = \frac{5 \text{ C. O.}}{0 \text{ N. C. O.}} = 100\,\%$

$\frac{Cd}{CD} = \frac{1 \text{ C. O.}}{4 \text{ N. C. O.}} = 20\,\%$ $\quad$ $\frac{De}{DE} = \frac{1 \text{ C. O.}}{4 \text{ N. C. O.}} = 20\,\%$

$\frac{Ce}{CE} = \frac{2 \text{ C. O.}}{3 \text{ N. C. O.}} = 40\,\%$ $\quad$ $\frac{Df}{DF} = \frac{2 \text{ C. O.}}{3 \text{ N. C. O.}} = 40\,\%$

$\frac{Cf}{CF} = \frac{3 \text{ C. O.}}{2 \text{ N. C. O.}} = 60\,\%$ $\quad$ $\frac{Ef}{EF} = \frac{1 \text{ C. O.}}{4 \text{ N. C. O.}} = 20\,\%$

If we assume that a single chromosome may have twenty-six factors arranged in a row, $A \ldots Z$, then

$\frac{Ab}{AB} = \frac{1 \text{ C. O.}}{24 \text{ N. C. O.}}$ $\quad$ $\frac{Az}{AZ} = \frac{25 \text{ C. O.}}{0 \text{ N. C. O.}}$

The cross-overs in which A alone is concerned would range from $4\,\%$ to $100\,\%$. In general we may say then that cross-overs and non-cross-overs would occur in this scheme, and that while the number of cross-overs increases with the distance apart, the number of non-cross-overs decreases. The percentage of cross-overs however is always proportional to the distance apart of the respective factors. If in addition to the chromosomes whose behaviour is traced in this scheme, there are other chromosomes which do not cross, the percentages of cross-overs become of course reduced, but nevertheless still remain exactly proportional to the distances apart of the factors.

This scheme, without further elaboration, appears at first sight to substantiate the crossing over hypothesis, and one is somewhat surprised to find that it has not been worked out in detail by Morgan himself.

Let us note that with n factors we get the following general formula for the series traced above:

$$\frac{1\text{ C. O.}}{(n-2)\text{N. C. O.}'}\quad\frac{2\text{ C. O.}}{(n-3)\text{N. C. O.}'}\quad\frac{3\text{ C. O.}}{(n-4)\text{N. C. O.}'}\cdots\frac{(n-1)\text{C. O.}}{(n-n)\text{N. C. O.}}.$$

The percentages of cross-overs become, using the formulae

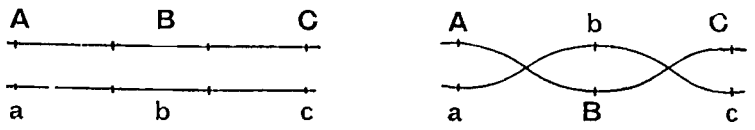
$$\frac{Ab\times 100}{AB+Ab}',\quad\frac{Ac\times 100}{AC+Ac}',\text{ etc.}$$

$$\frac{1\times 100}{n-1}',\quad\frac{2\times 100}{n-1}',\quad\frac{3\times 100}{n-1}',\cdots\frac{(n-1)\times 100}{n-1};$$

a series in arithmetical progression.

If the factors are uniform in size, the least difference between the observed percentages, that between Lethal 1 (·7) and White (1·0), may be regarded as the common difference, and the number of factors may be readily determined, as approximately 300. It is however very noteworthy (even critical for the crossing over hypothesis) that the highest percentage recorded in No. 1 chromosome is 65·5, and that the factors are crowded at one end of the chromosome and quite absent from the other. Why should one end of a chromosome be favoured more than the other? Surely the results should be of such a nature that they may be read from either end. The crowding represents high reduplication. Critical study of numerous cases of high reduplication will probably prove fatal to the Morgan hypothesis.

But a single-cross-over scheme such as this is inadequate to account for all the facts. With a single cross-over taking place by chance at any locus in the chromosome, we get all the combinations which appear in cases of dihybridism, i.e. where two pairs of allelomorphs, such as *Aa*, *Bb*, are involved, but we *do not* secure all the combinations which are required in a case of trihybridism, where there is another pair, say *Cc*. In order that such a combination as *AbC* should appear, even occasionally, it is necessary that a double cross-over should take place, thus:



Hence we may deduce the rule that if there are n pairs of segregating allelomorphs, the number of the loci for the crossings-over must be

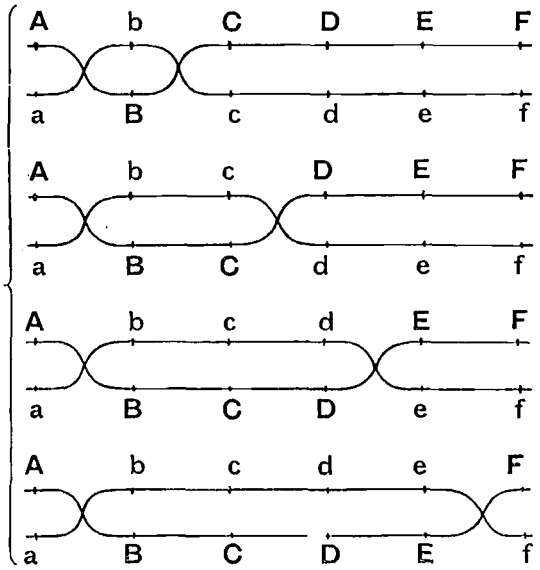
$n-1$. If crossing over is common to homozygotes and heterozygotes, as Sturtevant suggests, then the number of crossings over, whether segregation takes place or not should be only one less than the numbers of factors; in other words, there must be a crossing over place between each factor and its neighbours. Every possible assortment of characters is thus provided for.

But it does not seem probable that the law which applies to single cross-overs should apply also to double and multiple cross-overs, and indeed Morgan and his colleagues, in order to explain many remarkable deviations from the law, have already had to resort to three or more subordinate hypotheses, which they discuss in some detail under the heads of (1) differential viability, (2) double or multiple crossing over, (3) interference, and (4) incapacity for crossing over when the *Y* (male) chromosome is involved; etc.

I have thought it worth while to trace the effect of the addition of another crossing over in the case examined on pp. 284–286. If we take the figures on pp. 284–285 numbered 1 to 4, as a starting point, and confine our attention to those cross-overs in which *A* is involved, we get the following sets of figures and relations deduced from them.

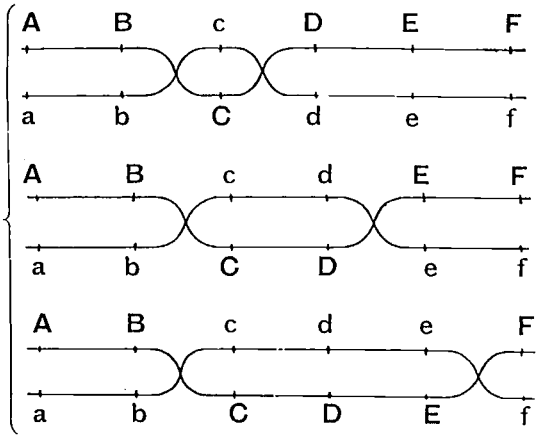
Double Crossing Over with 6 *Factors*, ABCDEF.

1.



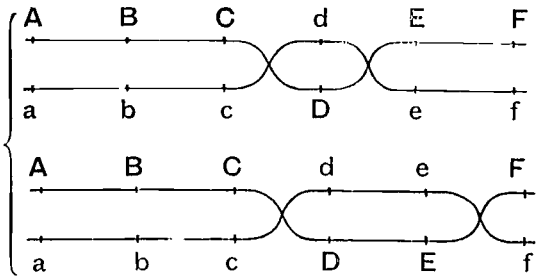
C. O.				N. C. O.			
Ab				*AC*	*AD*	*AE*	*AF*
Ab	*Ac*				*AD*	*AE*	*AF*
Ab	*Ac*	*Ad*				*AE*	*AF*
Ab	*Ac*	*Ad*	*Ae*				*AF*
4*Ab*	3*Ac*	2*Ad*	1*Ae*	1*AC*	2*AD*	3*AE*	4*AF*

2.



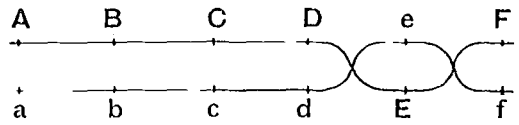
C. O.			N. C. O.				
Ac			*AB*		*AD*	*AE*	*AF*
Ac	*Ad*		*AB*			*AE*	*AF*
Ac	*Ad*	*Ae*	*AB*				*AF*
3*Ac*	2*Ad*	1*Ae*	3*AB*	0*AC*	1*AD*	2*AE*	3*AF*

3.



C. O.		N. C. O.				
Ad		AB	AC		AE	AF
Ad	Ae	AB	AC			AF
$2Ad$	$1Ae$	$2AB$	$2AC$	$0AD$	$1AE$	$2AF$

4.



C. O.	N. C. O.				
$1Ae$	$1AB$	$1AC$	$1AD$	$0AE$	$1AF$

Hence the sum of these cases will be

	C. O.				N. C. O.				
1.	$4Ab$	$3Ac$	$2Ad$	$1Ae$	$0AB$	$1AC$	$2AD$	$3AE$	$4AF$
2.		$3Ac$	$2Ad$	$1Ae$	$3AB$	$0AC$	$1AD$	$2AE$	$3AF$
3.			$2Ad$	$1Ae$	$2AB$	$2AC$	$0AD$	$1AE$	$2AF$
4.				$1Ae$	$1AB$	$1AC$	$1AD$	$0AE$	$1AF$
	$4Ab$	$6Ac$	$6Ad$	$4Ae$	$6AB$	$4AC$	$4AD$	$6AE$	$10AF$

The percentages of cross-overs become

$$\frac{Ab}{AB} = \frac{4 \text{ C. O.}}{6 \text{ N. C. O.}} = 40\,\% \text{ of cross-overs}$$

$$\frac{Ac}{AC} = \frac{6 \text{ C. O.}}{4 \text{ N. C. O.}} = 60\,\%$$

$$\frac{Ad}{AD} = \frac{6 \text{ C. O.}}{4 \text{ N. C. O.}} = 60\,\%$$

$$\frac{Ae}{AE} = \frac{4 \text{ C. O.}}{6 \text{ N. C. O.}} = 40\,\%$$

The series of percentages is a symmetrical one, but the terms are not in an arithmetical progression.

If we take twenty-six factors, A to Z, instead of six, A to F, we get the following series:

C. O.

1.	$24Ab$	$23Ac$	$22Ad$	$21Ae$	$20Af$	$19Ag$.....$1Ay$
2.		$23Ac$	$22Ad$	$21Ae$	$20Af$	$19Ag$.....$1Ay$
3.			$22Ad$	$21Ae$	$20Af$	$19Ag$.....$1Ay$
4.				$21Ae$	$20Af$	$19Ag$.....$1Ay$
5.					$20Af$	$19Ag$.....$1Ay$
6.						$19Ag$.....$1Ay$
⋮						⋮
24.						$1Ay$

The whole series of cross-overs in this case will be

$24Ab$	$46Ac$	$66Ad$	$84Ae$	$100Af$	$114Ag$
$126Ah$	$136Ai$	$144Aj$	$150Ak$	$154Al$	$156Am$
$156An$	$154Ao$	$150Ap$	$144Aq$	$136Ar$	$126As$
$114At$	$100Au$	$84Av$	$66Aw$	$46Ax$	$24Ay$

The number of N. C. O.'s is readily determined from the table on p. 292. The ratios $\left(\frac{\text{C. O.}}{\text{N. C. O.}}\right)$ are thus determinable in any case of double crossing over, and can be compared with the corresponding ratios for a single crossing over. (See Table on p. 293.)

Thus, with twenty-six factors, *A* to *Z*, a number approximating to those already located in the first chromosome, it is not difficult to calculate the corresponding series of ratios and the percentages by this method. The table on p. 293 gives the result of such a calculation arranged to shew the relationships of *single* and *double crossing-over*.

My mathematical colleague, Prof. Pinkerton, has suggested to me the following formulae, as a ready means of calculating the values of the ratio, $\frac{\text{C. O.} \times 100}{\text{C. O.} + \text{N. C. O.}}$.

Let there be n pairs of allelomorphs, Aa, Bb, Cc, etc., numbered from 1 to r and on to n, thus:

1	2	.....	r	..... n
A	B	.....	M	.....
a	b	.....	m	.....

The following formulae enable one to calculate the number of C. O.'s Am, etc., with little difficulty.

One crossing : $r-1$.

Two crossings : $(r-1)(n-r)$.

Three crossings : $(r-1)\frac{(n-r)(n-r-1)}{1\times 2} + \frac{(r-1)(r-2)(r-3)}{1\times 2\times 3}$.

Four crossings: $(r-1)\frac{(n-r)\ (n-r-1)\ (n-r-2)}{1\times 2\times 3}+\frac{(r-1)\ (r-2)\ (r-3)}{1\times 2\times 3}(n-r)$.

Five crossings: $(r-1)\frac{(n-r)\ \ldots\ (n-r-3)}{1\times 2\times 3\times 4}$

$$+\frac{(r-1)\ (r-2)\ (r-3)}{1\times 2\times 3}\times\frac{(n-r)\ (n-r-1)}{1\times 2}+\frac{(r-1)\ (r-2)\ (r-3)\ (r-4)\ (r-5)}{1\times 2\times 3\times 4\times 5}.$$

The following formulae give the numbers of C. O.'s + N. C. O.'s.

One crossing: $n-1$.

Two crossings: $\frac{(n-1)\ (n-2)}{1\times 2}$.

Three crossings: $\frac{(n-1)\ (n-2)\ (n-3)}{1\times 2\times 3}$. Etc.

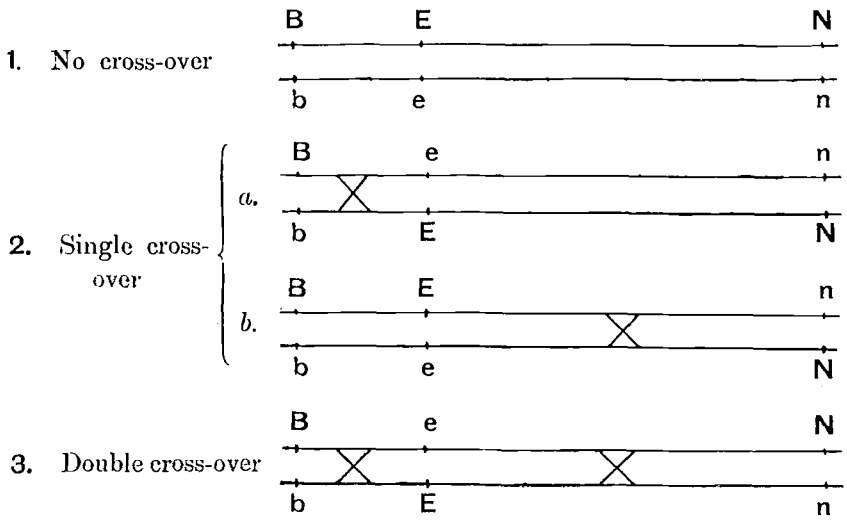
Non-Cross-Overs.

3 *AC*	*AD*	*AE*	*AF*	*AG*	*AH*	*AI*	*AJ*	*AK*	*AL*	*AM*	*AN*	*AO*	*AP*	*AQ*	*AR*	*AS*	*AT*	*AU*	*AV*	*AW*	*AX*	*AY*	*AZ*
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
22	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
21	21	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
20	20	20	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
19	19	19	19	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
18	18	18	18	18	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
17	17	17	17	17	17	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
16	16	16	16	16	16	16	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
15	15	15	15	15	15	15	15	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
14	14	14	14	14	14	14	14	14	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
13	13	13	13	13	13	13	13	13	13	0	1	2	3	4	5	6	7	8	9	10	11	12	13
12	12	12	12	12	12	12	12	12	12	12	0	1	2	3	4	5	6	7	8	9	10	11	12
11	11	11	11	11	11	11	11	11	11	11	11	0	1	2	3	4	5	6	7	8	9	10	11
10	10	10	10	10	10	10	10	10	10	10	10	10	0	1	2	3	4	5	6	7	8	9	10
9	9	9	9	9	9	9	9	9	9	9	9	9	9	0	1	2	3	4	5	6	7	8	9
8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	0	1	2	3	4	5	6	7	8
7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	0	1	2	3	4	5	6	7
6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	0	1	2	3	4	5	6
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	1	2	3	4	5
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	0	1	2	3	4
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	1	2	3
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	1	2
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1

	Double Crossing Over	Single Crossing Over
	Per cent.	Per cent.
$\frac{Ab}{\overline{AB}}$	$\frac{24}{276} = 8{\cdot}0$	$\frac{1}{24} = 4{\cdot}0$
$\frac{Ac}{\overline{AC}}$	$\frac{46}{254} = 15{\cdot}3$	$\frac{2}{23} = 8{\cdot}0$
$\frac{Ad}{\overline{AD}}$	$\frac{66}{234} = 22{\cdot}0$	$\frac{3}{22} = 12{\cdot}0$
$\frac{Ae}{\overline{AE}}$	$\frac{84}{216} = 28{\cdot}0$	$\frac{4}{21} = 16{\cdot}0$
$\frac{Af}{\overline{AF}}$	$\frac{100}{200} = 33{\cdot}3$	$\frac{5}{20} = 20{\cdot}0$
$\frac{Ag}{\overline{AG}}$	$\frac{114}{186} = 38{\cdot}0$	$\frac{6}{19} = 24{\cdot}0$
$\frac{Ah}{\overline{AH}}$	$\frac{126}{174} = 42{\cdot}0$	$\frac{7}{18} = 28{\cdot}0$
$\frac{Ai}{\overline{AI}}$	$\frac{136}{164} = 45{\cdot}3$	$\frac{8}{17} = 32{\cdot}0$
$\frac{Aj}{\overline{AJ}}$	$\frac{144}{156} = 48{\cdot}0$	$\frac{9}{16} = 36{\cdot}0$
$\frac{Ak}{\overline{AK}}$	$\frac{150}{150} = 50{\cdot}0$	$\frac{10}{15} = 40{\cdot}0$
$\frac{Al}{\overline{AL}}$	$\frac{154}{146} = 51{\cdot}3$	$\frac{11}{14} = 44{\cdot}0$
$\frac{Am}{\overline{AM}}$	$\frac{156}{144} = 52{\cdot}0$	$\frac{12}{13} = 48{\cdot}0$
$\frac{An}{\overline{AN}}$	$\frac{156}{144} = 52{\cdot}0$	$\frac{13}{12} = 52{\cdot}0$
$\frac{Ao}{\overline{AO}}$	$\frac{154}{146} = 51{\cdot}3$	$\frac{14}{11} = 56{\cdot}0$
$\frac{Ap}{\overline{AP}}$	$\frac{150}{150} = 50{\cdot}0$	$\frac{15}{10} = 60{\cdot}0$
$\frac{Aq}{\overline{AQ}}$	$\frac{144}{156} = 48{\cdot}0$	$\frac{16}{9} = 64{\cdot}0$
$\frac{Ar}{\overline{AR}}$	$\frac{136}{164} = 45{\cdot}3$	$\frac{17}{8} = 68{\cdot}0$
$\frac{As}{\overline{AS}}$	$\frac{126}{174} = 42{\cdot}0$	$\frac{18}{7} = 72{\cdot}0$
$\frac{At}{\overline{AT}}$	$\frac{114}{186} = 38{\cdot}0$	$\frac{19}{6} = 76{\cdot}0$
$\frac{Au}{\overline{AU}}$	$\frac{100}{200} = 33{\cdot}3$	$\frac{20}{5} = 80{\cdot}0$
$\frac{Av}{\overline{AV}}$	$\frac{84}{216} = 28{\cdot}0$	$\frac{21}{4} = 84{\cdot}0$
$\frac{Aw}{\overline{AW}}$	$\frac{66}{234} = 22{\cdot}0$	$\frac{22}{3} = 88{\cdot}0$
$\frac{Ax}{\overline{AX}}$	$\frac{46}{254} = 15{\cdot}3$	$\frac{23}{2} = 92{\cdot}0$
$\frac{Ay}{\overline{AY}}$	$\frac{24}{276} = 8{\cdot}0$	$\frac{24}{1} = 96{\cdot}0$
$\frac{Az}{\overline{AZ}}$	$\frac{0}{300}$	$\frac{25}{0} = 100{\cdot}0$

The maximum number of cross-overs in double crossing over occurs between A on the one hand, and M and N on the other. The number of cross-overs is no longer proportional to the distance apart of the factors. Y is twenty-four times the distance of B from A, but the cross-overs Ab and Ay are equal in number. C is twice as far from A as B is, yet $\frac{Ac}{Ab} = \frac{15{\cdot}3}{8{\cdot}0}$, i.e., is less than 2.

Now these two systems of crossing over obviously cannot occur together in the same chromosome and the same nucleus. The gametes consequently owe their constitution to the operation of one or the other scheme (not to both). The observed ratios can only be adequately interpreted if we know the relative frequency of the divisions which occur in each of these two systems. The American authors appear to have adopted the following point of view. If three pairs of factors are concerned, *Bb*, *Ee*, *Nn*, it is necessary, that all kinds of assortments should be obtained, that three types of behaviour should be possible to the chromosomes, as represented in the following diagram.

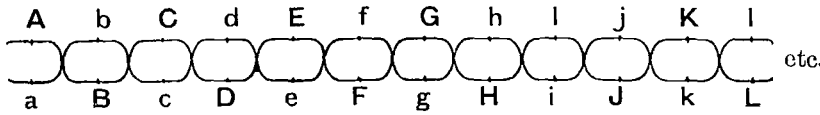


By experiment, the eight types of gametes postulated are actually found, as well as the *numbers in which they occur*, and those individuals whose constitution corresponds to the hypothetical double cross-over are always fewer in number than those of any of the other classes.

These three types of behaviour appear to be independent of each other, as by hypothesis, they take place in different nuclei. I think

therefore it would be an error to try and shew the possible mathematical relations which may exist between them. The frequency of a double cross-over can perhaps be calculated, like a double event, from the frequency of the corresponding single cross-overs, but it is extremely doubtful whether we are justified in assuming any mathematical relationship between a no cross-over (whole distance) and a single cross-over (part distance). But the numbers of the gametes alone, even were such mathematically related to each other, would not suffice to enable us to calculate the error of the ratio $\frac{\text{C. O.}}{\text{N. C. O.}}$ due to the presence of double crossing over. We should require to know at least the *total number of the factors* located in the chromosome as well.

But there are greater difficulties involved in the hypothesis. We have seen that there must be as many crossing over places as there are known factors, less one. By hypothesis, these factors are not recognisable unless they are separable in the process of crossing over. Hence we can fairly assume that multiple crossing over must be frequent; if the F_1 plant is heterozygotic for the twenty-six factors A to Z, there must be twenty-five crossing over places, and the chromosome would sometimes appear as a closely wound spiral, thus:



In such a scheme as this, with the maximum of crossing over, there are cross-overs and non-cross-overs in abundance, but for each type of cross-over, there is no corresponding non-cross-over. Crossing over is absolute, i.e. 100 °/₀, for

$$\frac{Ab}{AB} = \frac{Ad}{AD} \cdots \frac{Az}{AZ} = \frac{1 \text{ C. O.}}{0 \text{ N. C. O.}}.$$

Every alternate factor, too, appears to be absolutely correlated; there is no crossing over at all in Morgan's sense.

It can be shewn that for each type of crossing over—simple, double, triple, etc.—there is a different system of ratios; and as a number of these systems must be in operation in any one experiment, whether recorded or not, it is practically impossible to find the true percentage of cross-overs due to a single crossing over by deducting those C. O.'s and N. C. O.'s which are due to multiple crossing over. All that we can say positively is, that double crossing over as compared with single

crossing over, increases the percentage of C. O.'s up to the middle point of the chromosome, and decreases it from that point to the end.

The accurate location of the factors in the chromosomes by means of the empirical determination of the ratio $\frac{\text{C. O.}}{\text{N. C. O.}}$ appears therefore to be impossible. At best an approximation only can be secured, as the correction for multiple crossing over is, at any rate, in the present state of our knowledge, unascertainable.

The difficulties due to differential viability may also be practically insurmountable. Interference, or the hindrance to free crossing over caused by the proximity of the factors, cannot be effective in a case of single crossing over. In multiple crossing over, however, such hindrances may certainly occur. A theoretical estimate of them is impossible until we have at our disposal additional data such as the dimensions of the chromosomes and chromomeres at the actual moment of crossing over. In the figure on p. 295, there appears to be a maximum of interference. Crossing over is absolute between the first and second, interference is absolute between the first and third factors.

The crossing over hypothesis, simple enough at first sight, is in reality beset with extraordinary difficulties.

1. Two cardinal points of the hypothesis are: (*a*) that the distance apart of the factors is only directly determinable when two pairs of allelomorphs are alone involved, and (*b*) that the complete assortment of factors is impossible unless there are as many crossings-over (and schemes) as the factors, less one. As the crossing over is supposed to occur indiscriminately in homozygotes and heterozygotes, the actual ratios found can seldom have a simple relation to the pairs of allelomorphs whose behaviour is actually recorded. The disturbing effect of the remaining pairs must always be allowed for. It is perhaps impossible to arrange an experiment in which the F_1 individual is heterozygotic for two pairs of allelomorphs only.

2. When the number of pairs of allelomorphs is reduced to three—*Aa*, *Bb*, and *Cc*—and all other disturbing features of the hypothesis are set aside, a condition, which in practice is perhaps unattainable, we have to note that, by hypothesis, four kinds of behaviour are traceable to the same chromosome:

(*a*) *no crossing over*, when one of the homologous chromosomes is the *Y* chromosome. Most of the experimental data are based on this hypothetical behaviour.

(*b*) *no crossing over*
(*c*) *single crossing over* } when the *Y* chromosome is excluded.
(*d*) *double crossing over*

Why should a chromosome behave differently as in *b*, *c*, and *d*, or indeed in all four cases? Surely, the cells concerned, and their nuclei, are for all practical purposes indistinguishable from each other.

3. There is a logical fallacy underlying the whole of the crossing over hypothesis, as applied to the location of the factors. Let it be granted that under certain limited conditions the number of cross-overs is proportional to the distance of the factors from each other. It does not follow that the distance of the factors from each other is proportional to the number of the cross-overs. It may be true that "all men are fools"; it does not follow from this that "all fools are men."

In truth, the foundation on which the hypothesis of crossing over has been built up appears to be very unstable. Ruptures and recombinations take place without any adequate cause. Why should a chromosome break where it crosses another? If it break, why not remain broken? If it recombine, what regulates the recombination? What force secures the absence of crossing over where the *Y* chromosome is concerned? What determines the constant numerical relation between the no-ruptures, single ruptures, and double ruptures?

Finally, let it be noted that the graphic representation of the location of the factors is a type of representation common to every set of phenomena which can be expressed as percentages. The exponents of the reduplication hypothesis may very well accept the plans of the chromosomes, as graphic representations of the relative strengths of reduplication. For their purposes the diagrams must be read from the 50 °/₀ mark (no reduplication) towards the two ends. 90 °/₀ is indeed a graphic representation of − 10 °/₀ (repulsion).

In this analysis it was not thought worth while to pursue in detail other but somewhat petty difficulties, such as the presence of a spherical chromosome, with cross-overs associated with it; the probability of untwisting taking place in metakinesis; the difficulty of demonstrating a rupture and recombination by the existing cytological methods, and so on. It may be noted, however, that *Drosophila* with its few chromosomes, may shew an unusual amount of chromosome isolation and of reduplication (linkage and crossing over). Whatever may be the fate of their ingenious but, I fear, rather overworked hypothesis, students of genetics will continue to welcome the detailed contributions of these American authors.