CHAPTER XII

VARIATION IN LINKAGE

Crossing over is not absolutely fixed in amount, but is variable. This statement does not refer to variability in the number of crossovers due to random sampling, but to variability due to fluctuation in environmental conditions, or due to internal changes in the mechanism of crossing over itself. For example, it has been shown *that the amount of crossing over in Drosophila is different* at different temperatures, and it has also been shown that there are factors (genes) carried by the chromosomes themselves that affect the amount of crossing over. These questions, that have already been touched upon in other connections, may be taken up here in more detail.

The work of Plough on the influence of temperature on crossing over in *Drosophila,* that has already been utilized, was concerned with the influence of different temperatures on the number of crossovers obtained. It may be recalled that he found that when the eggs were subjected to a given temperature during a certain stage in their maturation the amount of crossing over that took place, as shown in the kinds of flies produced, was definite in the sense that the average results were predictable for each specific temperature, and that there are values for different temperatures which, when plotted, give the curve drawn in Fig. 56.

Further details of one of the experiments may serve to make its significance clearer. Three points (or loci) were made use of that involved three mutant genes (and their diagnostic characters, of course). Males, pure for the three mutant characters, black body color, purple eyes, and curved wings were crossed to wild-type females. The F_1 female produced in this way would be heterozygous for the three mutant factors involved in the cross. Such an F_1 female was then bred to a male pure for the three recessive genes, black, purple, curved; and her offspring were kept at a given temperature until they emerged as flies, and then if necessary for some days longer in order that as many eggs as possible might have matured under the specified temperature. Controls of sisters and brothers were made in each case and kept at average "normal" temperature. In the table that follows crossing over between black and purple is indicated as "1st crossover," and between purple and curved as "2nd crossover," and between both as double crossover.

Ten different temperatures were tested. At 5° C. the eggs did not hatch, and at 35° C. the females were sterile. In the seven intermediate temperatures the results were those recorded in the next table.

b — pr — c

Number	Temp.	Total	Female parents hatched at temperature indicated below						Weighted Value for b—pr Region
			Non-cross-over	1st cross-over	2nd cross-over	Double cross-over	1st cross-over	2nd cross-over	
							per cent	*per cent*	*per cent*
2	9°	995	643	95	218	39	13.5	25.8	13.6
3	13°	2,972	1,854	310	716	92	13.5	27.2	17.5
4	17.5°	2,870	2,021	189	610	50	8.3	23.0	8.2
5	22°	15,000	11,318	735	2,775	172	6.0	19.6	6.0
7	29°	4,269	2,993	315	898	63	8.8	22.5	8.7
8	31°	3,547	2,265	333	785	164	14.0	26.7	18.2
9	32°	4,376	2,701	513	984	178	15.7	26.5	15.4

At the two lower temperatures the crossover value is high, *i.e.,* little crossing over occurs. At the next three temperatures (17.5°, 22°, 29° C.) the crossing over value is much less, while at the last two temperatures 29° and

31° C., it is high again. The control values for sister flies, at normal temperature (22° C.), are given in the next table.

Controls—female parents hatched at 22° C.

1st cross-over	2nd cross-over	Total	Non-cross-over	1st cross-over	2nd cross-over	Double cross-over
per cent	*per cent*					
6.1	19.2	904	683	47	166	8
7.8	20.1	3,622	2,655	231	685	51
5.9	19.5	2,219	1,678	108	409	24
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5.9	20.3	4,822	3,608	231	927	56
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The figures given in this table were obtained as a control for the last results, and from these data the results of crossing over are reduced to the same scale. These weighted crossing-over values for the first regions give the curve drawn in Fig. 56. The curve begins at a high level and drops rapidly. The first maximum is reached at about 13° C., and then falls to 17.5° C., where the level remains nearly constant for ten degrees more (27° C.). It rises rapidly at about 28° and reaches a second maximum at 31° to 32° C. Afterwards it is seen to fall until sterility occurs at 35° C.

The temperature curve of crossing over seems to show that the phenomenon is not a simple chemical reaction, for if it were we should expect for every rise in 10° C. the amount of change in crossing over to be approximately tripled. It would appear, therefore, that the phenomena might be due to the physical state of the materials involved in crossing over. Plough calls attention to the similarity of this curve to that shown by the amount of contraction of a frog's muscle. Here there is an increase from zero to 9° C., when a maximum is reached. After this, the amount of contraction decreases, reaching a low point between 10° C. and 20°C. It then rises rapidly, reaching a higher maximum than the first at about 28° C., after which it decreases until rigor sets in at 38° C.

The results of crossing over between purple and curved gave similar results, but the "distance" here is so great that double crossing over complicates the results; therefore they need not, for the present, be analyzed further. Attempts to change the crossing over value by starvation, moisture, increase in fermentation of the food, iron salts, etc., gave no results that seemed significant. On the other hand, Bridges had already noted that a decrease in the amount of crossing over is found in second broods as compared with first broods—ten-day periods. What change in the environment is behind this "age" difference is not clear, but since most of the eggs pass through this early prematuration stage in the larvæ and some of them may reach the maturation stage in the pupa, it is possible that prevailing conditions in one or the other of these physiological states may be responsible for the difference between these states and those that prevail after the fly has hatched.

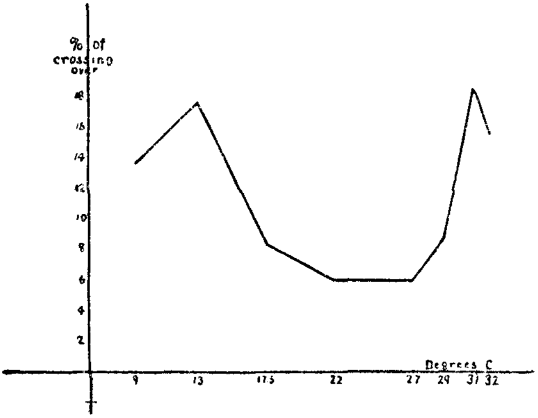

FIG. 56.—Curve showing influence of crossing over at different temperatures. (After Plough.)

Not only external factors but internal factors, and these genetic ones, may influence the amount of crossing over that takes place. Sturtevant has discovered two such genes in the second chromosome of a certain stock of *Drosophila*. A female from a wild stock from Nova Scotia was crossed to a male showing the characters vestigial and speck. One of the daughters was tested and gave no crossovers in 99 offspring, though the vestigial, speck hybrid usually gives about 37 per cent. of crossing over. All of the descendants of this female that were known, through linkage relations, to have the Nova Scotia second chromosome, gave the same result, while those of her descendants that did not have the particular chromosome did not show such a change in linkage. These relations held regardless of whether the chromosome involved had come from the father or the mother.

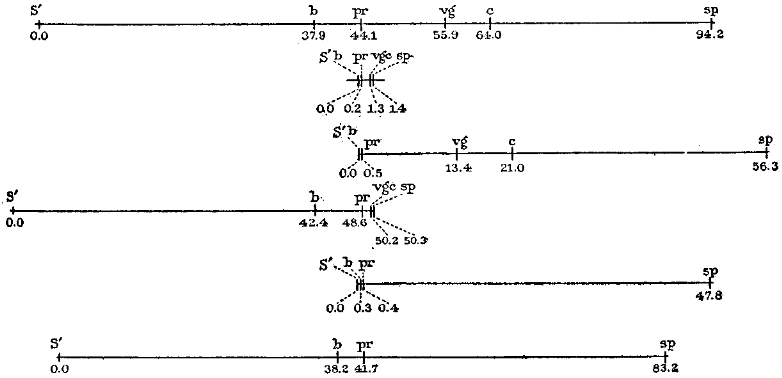

FIG. 57.—Diagram illustrating the effect on crossing over due to the presence of crossover genes. (After Sturtevant.)

A number of experiments were made with females having a Nova Scotia second chromosome, while the other second chromosome bore the mutant genes for black, purple, curved, and in other experiments other mutant genes were present. In Fig. 57 (upper line) all the genes studied, *viz.*, star (*S*), black (*b*), purple (*pr*), vestigial (*vg*), curved (*c*), and speck (*sp*) are indicated in their relative locations, *i.e.*, spaced in proportion to the usual amount of crossing over between them. Correspondingly, the short second line is based on the crossover relations of these factors when the female is heterozygous for the two Nova Scotia genes.

Further experiments were made with females (obtained by crossing over) in which only the "left half" of a Nova Scotia chromosome was present (third line), the other half being derived from an ordinary chromosome. The offspring of such a female showed that crossing over was decreased only in the left half.

When the right half of the Nova Scotia chromosome was present (fourth line) that half was "shortened." It follows that there are two (or possibly more) factors present, one in each half of the second chromosome of the Nova Scotia stock, each inhibiting almost completely crossing over in its own region, but not in the other region.

An equally surprising result was obtained from a female so constituted that the right halves of both members of this pair of second chromosomes were present, *i.e.*, when she was homozygous for the "right hand" pair of factors for little crossing over. Under these circumstances, the crossing over was normal for this end (last two lines). How such results are produced (quite aside from the nature of the factor producing them) is unknown. Almost inevitably, however, we think of the cause as a difference in the length or shape of the chromosome containing these factors, so that corresponding levels do not come together, hence failure of interchange. When, however, both chromosomes are affected in the same way their corresponding regions might be expected to come together and cross over.

The preceding results of Sturtevant's suggest the possibility that all genes may have an effect on crossing over—possibly one might think that in some mysterious way the crossing-over values shown by the genes are a function of their nature. It may be well to point out that in the only cases where the evidence suffices to give an answer to such a question, that answer is very clearly against such a view. For instance, if we determine the linkage between two factors *A–M* and then exchange one of the intermediate genes for its allelomorph, we find that in general the change has no effect on crossing over between *A* and *M*. If we exchange factors outside of *A* and *M*—either near them or far away—still no effect on crossing over between *A* and *M* is observed. If we substitute one allelomorph for another, in cases where more than two are known, we find no change in the crossing over for that level. This and other evidence shows that crossing over is quite independent of such genes, nevertheless there are other specific genes, as shown above, whose sole effect, or main effect at least, is to change the crossing-over values.

One highly important and significant result of Sturtevant's work on crossing-over factors should be noticed. *The order of the factors is not in any way changed by* the "shortening" process, as shown by the experiments in which three or more loci are followed at the same time.

The most remarkable fact connected with crossing over is that no crossing over at all takes place in the male of *Drosophila,* and this applies not only to sex-chromosomes (*XY*) but also to the other pairs or autosomes. When the absence of crossing over was discovered for sex-linked genes, it seemed probable that this was due to the presence of only one *X*-chromosome in the male, for at this time Steven's work had led us to conclude that the male *Drosophila,* like some other insects, is *XO*. Later, when failure to cross over in the male was found in other chromosomes as well, it was evident that some more general relation was behind the phenomenon in these chromosomes at least. It is true that other genetic evidence has shown that the *Y*-chromosome is "empty" (*i.e.*, contains no genes dominant to any of the mutant genes as yet discovered) and on this account one might still ascribe failure to cross over in this pair to its peculiar condition.

The interest in the situation became even greater when it was found that in the silkworm moth (in which the sex formula is reversed, so to speak) crossing over is again absent in the sex that is heterozygous for the sex factors—here the female. The female moth is apparently *ZW,* at least in two cases.

In one of the flowering plants, *Primula sinensis,* crossing over occurs in both sexes (Gregory, Altenburg), but the amount of crossing over in the pollen is somewhat different from that in the ovules. Gowen has examined Altenburg's data statistically and finds that the difference is probably significant.

That crossing over should take place in the sex that is homozygous for the sex-chromosomes (the female in *Drosophila,* the male in the silkworms) but in both sexual elements in the hermaphrodite plant (*Primula*) may appear to have a deeper significance, but more recent discoveries seem to deprive the results of any such meaning. Castle, for instance, gives data that show crossing over in the male rat (the male is probably heterozygous for the sex-chromosome), and Nabours gives data for crossing over in the male and female grouse locust, *Apotettix* (in which the male is presumably heterozygous). Until more cases are forthcoming it must seem doubtful, therefore, if any such relation as that mentioned above is a general one.