A CONTRIBUTION TO AN UNDERSTANDING OF CROSSING OVER WITHIN CHROMOSOME RINGS OF OENOTHERA

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There are two theories to account for linkage phenomena as observed in *Oenothera*. (1) Linked genes are always found within the same chromosome, that is, linkage is of the conventional or *Drosophila* type. The number of linkage groups is determined by the number of chromosomes in the genom, and bears no relation to chromosome configuration (SHULL 1928). (2) In addition to linkage of the ordinary type, genes may be distributed among several chromosomes and yet be linked, such linkage being induced by the cohesion of these particular chromosomes to form the circles so characteristic of *Oenothera*. The number of linkage groups is consequently determined by the number of chromosome groups, and varies from form to form (CLELAND 1923, 1924, 1926).

Neither of these theories is capable, unaided, of explaining all the facts. But while the difficulties in the way of "theory 1" would seem to be insuperable, an hypothesis supplementing "theory 2" has been advanced which seems to account satisfactorily for the few cases of exceptional behavior which constitute the only difficulty in the way of this theory (DARLINGTON 1931, EMERSON 1931). The present paper is devoted to a discussion of this supplementary hypothesis.

Leaving out of account the relation of the segmental interchange hypothesis to the problem of linkage (which hypothesis would definitely exclude "theory 1," if proved [CLELAND 1933]), "theory 1" meets its Waterloo in the phenomenon of linkage alteration described by RENNER (1925). It is impossible on the basis of "theory 1" to explain how two or more genes can be linked in one complex-combination and be independent in another. The painstaking and brilliant researches of RENNER leave no reason to doubt, however, that such alterations in linkage do occur. Furthermore, the investigations of CLELAND and OEHLKERS (1929, 1930), RUDLOFF (1929, 1930), GERHARD (1929) and EMERSON (1930, 1931) indicate clearly that these alterations are related to and accompany alterations in chromosome linkage. Genes which are linked when the chromosomes cohere into a large circle become independent when they come to lie in a hybrid in which the chromosomes are no longer organized into a large circle, but form several independent pairs. The alteration in linkage relations, therefore, and the correlation between linkage relations and chromosome con-

figuration are facts incapable of explanation by "theory 1," and sufficient in themselves to discredit the theory.

On the other hand, there are a few cases of exceptional behavior which seem opposed to "theory 2." According to this theory, the extent to which genes are linked must parallel the extent to which the chromosomes cohere into circles. *There must not be more linkage groups than there are* (not individual chromosomes in the genom, but) *chromosome groups*. While the cyto-genetic work cited in the previous paragraph shows that a close correlation does exist between the extent of genetic linkage and the extent of chromosome cohesion, nevertheless, a few cases are known in which there seem to be one too many linkage groups, and these constitute the difficulty in the way of "theory 2."

The supplementary hypothesis which seems to explain satisfactorily this apparent excess of linkage groups over chromosome groups has been presented by DARLINGTON (1931) and EMERSON (1931) who have suggested the possibility of ascribing the occurrence of segregation within a circle to *ordinary crossing over*. The argument of EMERSON is especially clear and convincing, being based upon actual experimentation with specific genes in *franciscana* and *franciscana sulfurea*.

When the present paper was first begun, it was with the purpose of extending this hypothesis in detail to those cases which have been cited repeatedly as exceptions to the chromosome cohesion theory and which have caused certain investigators to question its validity. These cases concern (1) the apparent existence in *Lamarckiana* (© 12, 1 pr.) of three linkage groups, as shown by SHULL; and (2) anomolous behavior of a flower size factor, analyzed by RENNER and others. When our manuscript was well under way, however, a valuable contribution by EMERSON and STURTEVANT (1932) appeared in which these matters are discussed at some length, making it unnecessary for us to discuss them further in detail. We have, therefore, eliminated a large portion of our manuscript, but have thought it well to retain and publish certain portions which summarize more completely than has hitherto been done the chief points in regard to the hypothesis, and which add certain points in regard to its application to the situation in *Lamarckiana*.

THE HYPOTHESIS

1. It is assumed that homologous end-segments of chromosomes pair in synapsis, no matter whether they belong to strictly homologous chromosomes or to chromosomes which are otherwise non-homologous. Whether these end-segments extend only a short way from the ends, as DARLINGTON (1931) suggests, or all the way to the spindle attachment, or their lengths vary in different chromosomes and in different complexes is not known,

but for the purpose of our argument the matter is unessential. (The length of homologous segments will determine to what extent pairing in *Oenothera* is to be called telosynaptic, parasynaptic, or semi-parasynaptic. If only very restricted regions at the ends synapse and the great bulk of the chromosomes remain unpaired in zygotene and pachytene, as DARLINGTON suggests, the chief contention of the early telosynaptists is supported in *Oenothera*, for the great bulk of the pachytene spireme would then be *univalent*. If, on the other hand, the homologous segments are long and the greater portion of the length of each chromosome enters into synaptic union with homologous portions of other chromosomes the situation could then be called without hesitation parasynaptic.)

2. To the extent to which pairing takes place, to that extent one might expect crossing over to occur. We may assume that crossing over may occur at any point along the paired segments and that it is of the same type as that found in paired chromosomes.

3. Crossing over would in no way affect chromosome configuration. It would, however, offer a mechanism whereby *complexes could exchange genes* and thus alter linkage relations.

4. The frequency with which a given gene in a circle chromosome becomes transferred to the opposite complex is dependent, not upon the distance from any other gene or group of genes as is true of paired chromosomes, but, as shown below, *upon its distance from the innermost (most proximal) point at which the segment in which it is found is paired with its homologue*. As will be seen in the accompanying diagram (figure 4), each end segment has a distal end which, in a sense, is free and a proximal end which is fixed by being attached to another and non-homologous segment. A gene located in such an end segment will be exchanged to the opposite complex by any single crossover occurring at any point proximal to it. (Assuming that crossing over occurs at the four strand stage, such a crossover will of course affect only two of the four strands.) The gene will, however, remain a part of its original complex if such crossovers as occur are distal to it. Clearly, therefore, genes which are more distally placed will stand a greater chance of being transferred to the opposite complex than will those placed nearer the proximal end of the chromosome segment. It follows that *the frequency with which a given gene is transferred to the opposite complex through crossing over will depend upon its distance from the innermost point at which the segment in which it is found is paired with its homologue*.

The occurrence of double crossing over will probably not affect the total frequency with which a given gene shows transfer from one complex to another. Double crossovers of three types may occur proximally to a given gene. In the first type, three of the four strands are involved in the two

crossovers (figure 1). The result, as far as the relation of the genes Aa to the complexes is concerned, is the same as when a single crossover occurs proximally to those genes, that is, in two of the strands the genes (A or a) are still associated with their original complex, but in the other two they are associated with the opposite complex. In the second type, the same two strands are involved in both crossovers. The two crossovers cancel each other, so far as affecting the relation of Aa to the complexes is concerned, so that genes Aa undergo no transfer (figure 2). In the third type, different pairs of strands are concerned in crossovers at different levels, with the result that all four strands become crossover strands so far as Aa are concerned, that is, A or a has become associated with the opposite complex in each strand (figure 3).

FIGURES 1-3.—Three types of double crossing over which may occur proximally to a given gene in a circle of chromosomes. Solid bars represent strands belonging to one "Renner complex," hatched bars those belonging to the opposing complex. Explanation in text.

If these three types of double crossing over occur with about the same frequency, it is probable that types 2 and 3 will neutralize each other in their effect on the relation of Aa to the complexes, giving a combined effect equal to the average action of single crossovers or of crossovers of type 1. We may then, for all practical purposes, speak as though only single crossovers occur proximally to a given gene, involving two of the four strands.

5. The *detection* of the transfer of a given gene to the opposite complex and the determination of the frequency of such an occurrence are usually complicated by the presence of balanced lethals in the circle. Owing to their presence, recessive genes are prevented from expressing themselves *except* in the case of a crossover. It is the percentage with which a *recessive type appears after selfing*, therefore, that makes it possible to determine the percentage in which the gene responsible for this type is transferred from one complex to the other.

Ability to use the backcross of the heterozygote to the recessive in determining crossover percentage will depend upon the forms used. When the heterozygote is an isogamous form like *Lamarckiana* and the recessive is the same form, the backcross cannot be used, for the impossibility of the survival of anything but complex-heterozygotes from this cross because of the lethals means that a 1:1 ratio will result, irrespective of the percentage in which crossing over occurs (leaving out of consideration such modifying influences as pollen and gone competition). Thus, a 1:1 ratio in backcross is actually obtained in the cases of genes which show such widely divergent ratios after self-pollination as 10:1 (*sulfurea*) and 3:1 (*brevistylis*) (SHULL 1926). In the case of a heterogamous form, however, the backcross can theoretically be used to test crossover percentage.

The difference in this respect between isogamous and heterogamous forms may be made clearer by the following examples in which α and β are gametes and G and g are genes carried by them. Crossover gametes and progeny are enclosed in brackets.

ISOGAMOUS SPECIES

Gametes of heterozygote		Gametes of homozygote		Resultant progeny
αG	$\times$	βg	$=$	$1:1$ $\left\{ \begin{array}{l} \alpha\beta Gg \\ \alpha\beta gg \end{array} \right.$
βg	$\times$	αg	$=$	$1:1$ $\left\{ \begin{array}{l} \alpha\beta Gg \\ \alpha\beta gg \end{array} \right.$
(αg)	$\times$	βg	$=$	$1:1$ $\left\{ \begin{array}{l} (\alpha\beta gg) \\ (\alpha\beta Gg) \end{array} \right.$
(βG)	$\times$	αg	$=$	$1:1$ $\left\{ \begin{array}{l} (\alpha\beta gg) \\ (\alpha\beta Gg) \end{array} \right.$

(Theoretical ratio of $\alpha\beta Gg$ to $\alpha\beta gg$ in progeny = 1:1)

HETEROGAMOUS SPECIES

Eggs of heterozygote		Sperms of homozygote		Resultant progeny
αG	$\times$	βg	$=$	$\alpha\beta Gg$
(αg)	$\times$	βg	$=$	$(\alpha\beta gg)$

(Theoretical ratio of $\alpha\beta Gg$ to $\alpha\beta gg$ in progeny = ratio of αG eggs to αg eggs)

In the following table are given the theoretical percentages in which recessive types may be expected to appear as a result of selfing in forms

TABLE 1

PERCENTAGE OF SPOROCTES IN WHICH CROSSING OVER OCCURS PROXIMAL TO Aa	RATIO OF A TO a AFTER SELFING
10	20.0 $A:1a$
20	10.10 $A:1a$
40	5.25 $A:1a$
50	4.33 $A:1a$
60	3.76 $A:1a$
80	3.17 $A:1a$
100	3.00 $A:1a$

with balanced lethals, following certain specific crossover percentages. (It is assumed that crossing over occurs at the four strand stage. If it takes place at the two strand stage, only one-half the amount of crossing over will be necessary to produce a given percentage of segregation after selfing.)

From this table it is evident that the occurrence of crossing over proximally to a given gene in 60 percent or more of the cases will result theoretically, after selfing, in an approximation to the 3:1 ratio in the next generation; and even as low as 40 percent will give a ratio that might be taken as 3:1 in small pedigrees (leaving out of consideration such modifying influences as pollen and gone competition).

It may be thought that 40 percent is a high crossover figure, but it must be reemphasized that we are not dealing with the percentage of crossing over between two genes, but rather with the percentage of cases in which *crossing over occurs at any point proximal to a given gene*. It therefore makes no difference at what point the crossing over between segments occurs, so long as it is proximal to the gene. It is clear that, in the case of a gene at the extreme distal end of a segment, crossing over will result in a transfer of this gene *every time* it (crossing over) occurs in this segment.

While we do not know how long the terminal segments are in any of the chromosomes of *Oenothera*, it is quite probable that they are long enough to allow for at least one crossover in 60 percent of the cases, certainly in some of the segments. Indeed, if DARLINGTON'S crossing over hypothesis be correct, according to which every chiasma represents an antecedent crossover, the average number of crossovers per segment must be somewhat in excess of one (DARLINGTON 1931). The chances are good, at any rate, that terminally placed genes will show, after selfing, a percentage of transfer which will result in an apparent independence of these genes from their complexes. Genes not terminally placed, but lying farther back from the end, will show a corresponding reduction in the percentage of transfer and will segregate out less frequently.

Application of this hypothesis to the situation in O. LAMARCKIANA

SHULL has shown the apparent presence of three independent gene groups in *Oe. Lamarckiana* as follows:

GROUP 1	GROUP 2
<i>sulfurea</i> (<i>s</i>) = sulfur flowers <i>angustifolia</i> } <i>stenophylla</i> } = narrow-leaved forms	<i>brevistylis</i> (<i>br</i>) = short style
GROUP 3	
<i>nanella</i> (<i>n</i>) = dwarf stature <i>pervirens</i> (<i>p</i>) = all green <i>Rubricalyx</i> (<i>Rh</i>) = red hypanth. <i>green hypanthium</i> (<i>R^c</i>) <i>funifolia</i> (<i>f</i>) = revolute leaves	<i>vetaurea</i> (<i>v</i>) = old-gold flowers <i>supplena</i> (<i>sp</i>) = double flowers <i>bullata</i> (<i>bu</i>) = crinkly leaves <i>Red-vein</i> (<i>Rv</i>) = red midribs of leaves

Rubrifolia (*Rf*) = red on leaves
 zygote lethal 1₁
 zygote lethal 1₂
 (possibly gamete lethals)

acutifolia (*af*) = acute, nearly
 uncrinkled
 leaves

The material showing these linkage relations has had, in some cases at least, a circle of twelve and one pair, and probably has possessed this configuration in most of the cases. If the pair be made to account for one of the linkage groups, the remaining two groups are necessarily placed within the circle of twelve chromosomes. This raises the question as to whether it is possible for the circle to be the basis for two linkage groups and, if so, which groups are to be assigned to the circle and which to the pair.

In the first place, it is logical to assume that group 1 is associated with the circle of twelve since it is the largest group. With respect to the other two groups, EMERSON and STURTEVANT (1932) have clearly shown that it is group 3 which belongs to the pair and that *brevistylis*, the sole member of group 2, belongs, in reality, in the circle along with the genes of group 1.

The genes which are located in the circle of chromosomes in *Lamarckiana* are probably situated at different loci along their respective segments. It is extremely improbable that all known genes belonging to the circle lie either so close to the proximal ends of their segments that they never segregate, or so close to the distal ends that they seem to be independent of the complexes. It is reasonable to suppose, therefore, that the segregation frequencies of circle genes which have come to the attention of the *Oenothera* workers will not be the same. Unfortunately, the list of known genes governing visible characters is not large and crossing over data are scanty. Nevertheless, the following data in regard to segregation frequencies of these genes indicate that they lie at varying distances from the distal ends of their segments.

Rubrifolia, which appeared as a dominant mutation, has given, by selfing, progenies consisting entirely of *Rubrifolia*. These plants, however, have been shown to be heterozygous, since backcrossing to the recessive type gave segregating progenies (new data). A single possible case of crossing over has been found involving this gene. In a family of 51 individuals from the selfing of *Rubrifolia* one green rosette appeared (SHULL, private correspondence). The gene *Rf* no doubt lies very close to the proximal end of the segment.

Nanella, a recessive gene, segregates out from *Lamarckiana* in about 0.5 percent of the population, a phenomenon probably due to crossing over. Its locus is apparently quite close to the innermost point at which its segment is paired with its homologue.

Pervirens appeared originally as one plant in a family of 79 (SHULL 1921). From data of SHULL (1932), it appears that this factor segregates out very rarely after selfing a plant heterozygous for it. A summarization

FIGURE 4.—The relative (not absolute) positions of various genes in the circle of *Lamarckiana* based upon crossover ratios. Data of EMERSON (1931) indicate that *p*, *n*, and *s* are in the same chromosome, possibly in the same segment. Data of EMERSON and STURTEVANT (1932) indicate that *Co* and *br* are also in a single chromosome, possibly in the same segment. Genes are placed in separate chromosomes in the absence of data to the contrary. The relative positions of *Co* and *br* are unknown. It is also unknown to which complex most of the genes belong. Black and hatched chromosomes belong to opposing complexes.

of the data in tables 1, 3, and 4 of SHULL (1932) shows a total of but 9 *pervirens* crossovers in a total of 4073 plants derived from selfing plants heterozygous for *pervirens*. This suggests that the *pervirens* gene lies so close to the inner end of its segment that crossing over occurs very rarely at loci proximal to it.

There is strong reason to believe that *Rubricalyx* and red cone are alleles of *pervirens*. Either that, or they are very closely linked. One would expect them all to show, therefore, about the same percentage of crossing over. In the absence of data in this regard, no further statement can be made.

Stenophylla arose in 1925 from the trisomic mutant *Oe. liquida* (DE VRIES 1929). It has never arisen directly from *Lamarckiana* itself, either in the experience of DE VRIES or in that of SHULL (private correspondence) who received the strain from DE VRIES. It is evident that the gene for this character resides close to the proximal end of its segment. It is even possible that the segment in which it lies is longer than its homologue in *Lamarckiana* and hence that crossing over of this gene is impossible in straight *Lamarckiana*.

Angustifolia arises almost every year from *Lamarckiana* in the experience of DE VRIES (1929). SHULL, whose material came from DE VRIES, finds also that this form appears occasionally in his *Lamarckiana* cultures (private correspondence). No ratios have been given, but it is evident that this gene lies far enough out on its segment to be crossed over with fair frequency to the opposite complex.

Funifolia. This character appeared originally in five plants in a family of 100 after selfing a plant which was presumably heterozygous for this gene (SHULL 1921). It has not subsequently appeared, inasmuch as the line from which it sprang has not been carried on. The appearance, however, of several plants bearing the new recessive character suggests that they arose by crossing over, and that the *f* locus is far enough removed from the most proximal point of pairing of its segment to make crossing over proximal to this gene possible in something like 10 percent of the sporocytes, thus resulting in segregation of the recessive type in approximately 5 percent of the population.

Sulfurea segregates out in selfed line in a ratio of about 10:1, as shown by SHULL (1926). He obtained a total of 943 non-*sulfurea* to 94 *sulfurea*. In backcrosses, however, the 1:1 ratio was approximated, there having been produced a total of 514 non-*sulfurea* to 508 *sulfurea*. This would indicate that *s* lies at such a distance from the inner end of its segment that crossing over occurs proximally to it in about 20 percent of the sporocytes, resulting in a 10 percent segregation of the recessive condition.

Brevistylis. A summary of SHULL's data (1926, 1928) shows *brevistylis* to have appeared, upon the selfing of heterozygotes, in 47 plants out of 635, or in the ratio of 3.32:1. The locus of *br* is close enough to the distal end of its segment so that crossing over occurs proximally to it in from 75 percent to 100 percent of the sporocytes, resulting in an approximation to a 3:1 ratio.

To these genes must undoubtedly be added, as has been shown by EMERSON and STURTEVANT (1932), another factor, one for flower size, which has an extremely distal position in its segment.

These data are, on the whole, meagre. Nevertheless, they furnish some evidence that the loci of the various genes are differently placed in relation to the ends of their respective segments, some lying close to the proximal ends, others in intermediate positions, still others close to the distal ends. The relative (not absolute) positions of these genes are schematically represented in the accompanying diagram (figure 4), it being tentatively assumed where there is absence of evidence to the contrary that they lie in different segments. On the whole, it seems reasonable to postulate that the varying degrees with which recessive genes belonging to group 1 become detached from their lethals and segregate out is due to their being located at varying distances from the proximal ends of their segments. The genes *br* (*brevistylis*) and *Co* (flower size) merely represent, therefore, the extreme in a series of genes in group 1 which show more or less segregation from their lethals.

CONCLUSION AND SUMMARY

In conclusion, we may say that the suggestion made independently by DARLINGTON and EMERSON that crossing over might occur between the paired strands of the end-segments of *Oenothera* chromosomes, transferring genes situated distally to the crossover to the opposite complex, may be applied to those cases which have been cited specifically in opposition to the chromosome cohesion theory. If this hypothesis be true, cause for controversy among proponents of opposing theories of linkage in *Oenothera* disappears. It is evident that chromosome cohesion results in the linkage of genes situated in different chromosomes. It is probable, however, that one or more genes lying close to the distal ends of their respective segments are transferred by crossing over to the opposing complex with frequencies sufficient to render them apparently independent of their complexes.

Unfortunately, the number of known genes is too small to make feasible genetical tests of this hypothesis. Such tests must await the accumulation of a larger number of genes. There can be little doubt, however, that, as matters stand, the chromosome cohesion theory supplemented by the crossing over hypothesis constitutes by far the most satisfactory explanation of genetic behavior in *Oenothera*, and the only explanation that does not go counter to known facts.

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