Chromosome Ring Formation in *Rhoeo discolor*

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Received August 12, 1931

BELLING's (1927) suggestion that chromosome ring formation in *Oenothera* is caused by segmental interchange between non-homologous chromosomes has received strong support during the past few years. This theory applied to the situation found in *Datura* and *Oenothera* by BLAKESLEE and CLELAND seems to meet all requirements, and since predictions of chromosome behavior in *Oenothera* hybrids are in accord with the theory there seems to be little doubt of its validity.

According to the theory of segmental interchange no two chromosomes in a closed ring or complete chain should be identical and at least half of them are composed of segments from two non-homologous chromosomes. Any two adjacent chromosomes may be morphologically different and must be different in their genetic constitution. The order of the individual chromosomes in the ring or chain should always be the same. The chromosomes are so similar in *Oenothera* that the individual chromosomes cannot be identified, although their consistent order in the ring is indicated by the genetic investigations.

In *Rhoeo discolor* the twelve somatic chromosomes differ considerably in length and position of the spindle fiber attachment and it should be possible to determine the sequence of the chromosomes in the chain at meiosis. BELLING (1927) described ring formation in *Rhoeo* but did not make a detailed study of chromosome size or behavior. DARLINGTON (1929) made a more complete study of *Rhoeo* chromosomes and found four somatic chromosomes with subterminal constrictions. At metaphase of the first meiotic division he found the chromosomes in rings, in one chain, two chains or in some cases three chains. Various types of non-disjunction were described. In none of his dozen figures of metaphase chromosomes is it possible to identify all of the four heterobrachial chromosomes although one or two can usually be recognized. KATO (1930) also has made an extensive study of *Rhoeo* chromo-

somes. Both he and DARLINGTON have described non-disjunction and occasional lagging chromosomes. KATO found the following distribution of the chromosomes at the first reduction division: 6:6 (64%), 5:7 (33%), 5:2:5 (1%), and 5:1:6 (1%). According to KATO the order of the chromosomes is: two heterobrachial chromosomes, four isobrachial chromosomes, two heterobrachial chromosomes and four isobrachial chromosomes. The heterobrachial chromosomes are said to pair either at their proximal ends or their distal ends, although KATO recognizes that this interpretation is not in accord with the theory of segmental interchange. KATO's diagrams are not supported by his drawings which show little or no individuality of the chromosomes.

When the chromosomes of *Rhoeo discolor* are properly fixed and stained it is possible to identify at least five of the twelve chromosomes at meiosis, and with more refined technique it is possible that all of them can be differentiated.

Most of the material for this study was collected at the Harvard Botanic Gardens at Soledad, Cuba, but plants were also obtained from several sources in the United States. The writer is greatly indebted to Professor OAKES AMES, Supervisor of the Arnold Arboretum and the Harvard Botanic Garden, for making possible the trip to Cuba.

The somatic chromosomes were obtained from root tips fixed in NAVASCHIN's solution and in modification of FLEMMING's solution. All of the preparations were stained with crystal violet iodine.

The somatic chromosomes of *Rhoeo discolor* are shown in Fig. 1. After examining a considerable number of division figures this one was found where all of the chromosomes lie in the same plane so that the drawing shows approximately the relative lengths of the individual chromosomes. Four of the somatic chromosomes are constricted near the ends and will be referred to as the heterobrachial chromosomes, several show an approximately median constriction, while the others are heterobrachial but not so extreme as the first type mentioned. Some of the chromosomes appear to be morphologically homologous but there is a rather distinct lack of association of similar chromosomes compared with the somatic figures in the closely related genus *Callisia*, where only bivalents are formed at the first meiotic division. In Fig. 1 it will be observed that chromosome *cD* is unlike any of the other three isobrachial chromosomes and that chromosomes *Cb* has no homologue. The seven chromosomes which are lettered are those which can easily be identified in the meiotic divisions although it is not possible to differentiate each of the two adjacent heterobrachial chromosomes in the

ring. According to the segmental interchange theory no two somatic chromosomes should be alike, but if equal interchange occurred between non-homologous chromosomes of similar morphology, the two segmental interchange chromosomes would be similar in appearance.

The 12 chromosomes of *Rhoeo* are usually arranged in a ring, or one or two chains, at "diakinesis" of the first meiotic division. The proportion of rings or single chains is apparently dependent to a considerable extent on environmental conditions, so no detailed study was made of the frequency of the different types of chromosome association. When properly fixed and stained it is easy to count not only the twelve chromosomes in the ring, but also to see the point of spindle fiber attachment in each chromosome so that the 24 chromosome segments can be recognized.

The closed ring of 12 chromosomes is clearly shown in Fig. 2. The heterobrachial chromosomes are invariably united in twos by their proximal ends and the two pairs thus formed are invariably connected to each other by a large chromosome. The large connecting chromosome is not strictly isobrachial, but one end *b* is somewhat longer than the other end *C*. The heterobrachial chromosome *Cc* is somewhat longer than the others and is invariably attached to the *C* end of the connecting chromosome *Cb*. Often a short isobrachial chromosome, *DE*, can be identified to the left of *Dc*, followed by a third heterobrachial "pair" *Ee* and *ed*, which are also united by their proximal ends. The arrangement of these two connecting chromosomes and the three "pair" of heterobrachial chromosomes is always the same. Another chromosome ring showing the chromosome sequence is shown in Fig. 3.

Chromosome chains are more frequently found than the rings. Several types of these chains are shown in Figs. 4, 5, and 6. In all of these chains the sequence of the chromosomes is the same as that found in the rings, although it was not always possible to identify the third heterobrachial pair of chromosomes. The segmentation of the chromosome ring was found to be due to the detachment of the ends of the chromosomes and in no case was it due to breaks at the point of spindle fiber attachment. One might expect the breaks to occur most frequently between the short arms of the heterobrachial chromosomes, but in these figures the breaks have occurred between the longer segments.

In about half of the first meiotic divisions observed the alternate chromosomes in the rings pass to opposite poles. A typical case is

Fig. 1. The 12 somatic chromosomes of *Rhoeo discolor*. Four chromosomes are distinctly heterobrachial. Figs. 2 and 3. A ring of 12 chromosomes at "diakinesis." The spindle fiber constriction of each chromosome is easily recognized. The normal heterobrachial chromosome *Cc* is always associated with the interchange chromosome *cD* by the proximal ends as are the heterobrachial chromosomes *bB* and *Ba*. Chromosome *bC* invariably connects these two heterobrachial "pairs." Chromosome *DE*, and the heterobrachial "pair" *Ee* and *ed* can also often be identified, and all of these chromosomes are invariably in the same order in the ring. Figs. 4, 5 and 6. Chromosome chains showing the order of the chromosomes. Breaks occur only between chromosomes, never at the fiber constrictions.

shown in Fig. 7. It is more difficult to identify the individual chromosomes at metaphase, but the heterobrachial pairs can usually be distinguished and are indicated in the figures.

Non-disjunction is very common in *Rhoeo* and the irregularities are greater than the chromosome counts at telophase would indicate. An example of double non-disjunction is shown in Fig. 8. If chromosome

Fig. 7. First metaphase showing alternate chromosomes passing to opposite poles. The heterobrachial chromosomes can be identified at this stage. Fig. 8. Double non-disjunction on the same side of the spindle. Fig. 9. Double non-disjunction on opposite sides of the spindle. Such types of non-disjunction will result in the same number of chromosomes at each pole but each gamete will be deficient for a chromosome segment and will probably be lethal.

bC and *Cc*, and the two directly beneath, all pass to the same pole two pollen grains would receive 7 and the other two would receive 5 chromosomes. Such distributions are occasionally observed at telophase. If, however, chromosome *aB* passes to the opposite pole then all four pollen grains will receive 6 chromosomes, but since chromosome deficiencies would exist in all of the microspores the pollen grains would be sterile, even though they contained the normal chromosome number. Double non-disjunction on opposite sides of the ring has also been observed and an example is shown in Fig. 9. Each of the four microspores would apparently have received 6 chromosomes, but the pollen grains would be sterile due chromosome deficiencies. For example the chromosomes passing to the upper pole will contain no chromosome segment *C* while those passing to the lower pole will contain no chromosome segment *D*.

The chromosomes retain their individuality during interkinesis and the sister chromatids are associated only at the point of the spindle fiber attachment. The second division is normal and regular tetrads are formed. The first division in the microspore can often be observed but sufficient figures were not obtained for an adequate frequency

distribution of the different types of chromosomes. The 6 gametic chromosomes in a microspore are shown in Fig. 10. Two distinctly heterobrachial chromosomes can be recognized.

Fig. 10. The gametic chromosomes in the first division of the microspore.

Although normal tetrads are formed, about 80 to 90 percent of the pollen grains are morphologically imperfect. The plants are partially fertile and several seeds are set in each bract.

When the meiotic chromosomes are stained lightly it is possible to see the coiled chromonemata, but in such preparations it is more difficult to determine the points of spindle fiber constrictions.

Non-conjugation of chromosomes

In the spring of 1930 the writer obtained cuttings of *Rhoeo discolor* from Dr. R.A. HARPER at Columbia University. Six plants blossomed in the late summer and an examination of the pollen mother cells showed essentially the same condition as found in the Cuban material. Chromosome rings and chains were invariably found at meiosis in all plants. During the fall of 1930 the green house temperature dropped to about 45 degrees Fahrenheit for several days. After this cold period an examination of these same plants showed a complete lack of pairing of the chromosomes. A typical case of asynapsis is shown in Fig. 11.

11

12

Fig. 11. The 12 univalent chromosomes at the first "meiotic" division. Asynapsis has been caused by low temperatures. Fig. 12. The 12 chromosomes at the second "meiotic" division following asynapsis.

The 12 chromosomes at the first "meiotic" division show no evidence of pairing and each chromosome is split lengthwise. These chromosomes divide equationally so that each diad receives 12 chromosome. A second equational division also occurs so that tetrads are formed and each microspore contains the complete number of somatic chro-

mosomes. A second division is shown in Fig. 12. If such diploid gametes are produced in megasporogenesis it should be possible to obtain tetraploid individuals with only bivalents at the first meiotic division. Seeds from cold treated plants have been planted to determine if such tetraploids can be produced.

Later in the year of 1930 additional plants were kept in the Arboretum cold pit where the temperature was about 36 degrees Fahrenheit. Only a few of the pollen mother cells showed complete asynapsis under these conditions. The others in the same anthers showed the usual rings and chains although they were perhaps somewhat more irregular than those found in the control plants.

When the cold treated plants were again grown under normal conditions for some time they no longer showed any evidence of asynapsis.

Discussion

Complete rings involving all of the meiotic chromosomes have been described in species of *Oenothera*, *Rhoeo* and *Hypericum*. Rings of four or more chromosomes have been found in more than half a dozen genera, of which those of *Datura*, and *Zea* have been studied most carefully. Recently STURTEVANT and DOBZHANSKY (1930) have presented genetic evidence for segmental interchange between non-homologous chromosomes in *Drosophila*, which presumably results in a ring of four chromosomes at meiosis.

According to the theory of segmental interchange an exchange of segments occurs so that in a ring of four chromosomes, *Aa* and *Bb* are the original chromosomes, and *Ab* and *aB* are the interchange chromosomes. In the discussion and in the diagrams a capital letter represents the left end of a chromosome and a small letter the right end of a chromosome. For instance, *Aa* or *Cc* represent normal chromosomes while *Ab* is a chromosome consisting of the left end of *Aa* and the right end of *Bb*, and *AC* is a combination of the left ends of *Aa* and *Cc*.

A study of simple rings of four chromosomes has shown that different types of segmental interchange can occur and that in some cases more than two gametic combinations of chromosomes can be produced.

In *Datura* a ring of four chromosomes is obtained by crossing "b" race with a standard line (BLAKESLEE and CLELAND 1930). If *Aa* and *Bb* represent the two chromosomes in the standard line which have produced *Ab* and *aB* in the "B" line then the chromosome ring in the hybrid would consist of *Aa-aB-Bb-bA*. The two A segments would,

of course, be united to form a closed ring. Since the hybrids are fertile, apparently only two classes of gametes are produced: $Aa-Bb$ and $Ab-aB$. Presumably the alternate chromosomes in the ring always pass to opposite poles. Both classes of gametes should be produced in equal numbers, and in the F_2 half of the progeny should have chromosome rings.

Rings of four chromosomes have also been found in *Zea* (BURNHAM 1930) and one of these has been studied in considerable detail by Miss MCCLINTOCK (1930). Miss MCCLINTOCK's figures of the early pairing of the chromosomes are remarkably clear. If we designate the two standard chromosomes involved as Aa and Bb then the segmental interchange chromosomes are Ab and Ba . The left end of Aa has united with the right end of Bb to produce Ab while Ba is composed of the right end of Aa and the left end of Bb . A study of microspore divisions showed that six types of gametes are produced: $Aa-Bb$, $Ab-Ba$, $Aa-Ab$, $Aa-aB$, $aB-Bb$, and $Ab-bB$. The first two types should be fertile since they carry a complete haploid complement, while the others should be sterile. Miss MCCLINTOCK concludes that since the sterility is 50%, that in half of the sporocytes any two adjacent chromosomes in the ring go to the same pole, forming sterile combinations, and in the other half of the sporocytes the adjacent members go to opposite poles, forming fertile combinations.

In the chromosomes of *Zea* described by Miss MCCLINTOCK and in those described by COOPER and BRINK (1931) the segmental interchange has not occurred at the point of spindle fiber attachment as usually seems to be the case in *Datura* and *Oenothera*. In both cases the breaks have occurred towards the distal ends of the two homologous chromosomes.

It is also evident, from the chromosome behavior in *Zea*, as clearly shown by Miss MCCLINTOCK and by COOPER, that the homologous segments of the four chromosomes are paired throughout their length. According to DARLINGTON (1929) in his discussion of chromosome rings, "it is just the constitution of the end of the chromosomes that would govern the metaphase association of the whole." BELLING and BLAKESLEE have expressed similar views. The available evidence indicates, however, that homologous segments of chromosomes have an equal attraction along their entire length, but that the formation of terminal chiasmata or the terminalization of median or subterminal chiasmata is responsible for the attachment of the chromosomes only at their ends at the first meiotic metaphase. Terminal chiasmata are

often found in the bivalents of *Zea* although the chromosomes are completely synapsed at earlier stages and crossing over is apparently not limited to the extreme ends of the chromosomes.

STURTEVANT and DOBZHANSKY (1930) have also obtained translocations in *Drosophila* which are similar to the cases of segmental interchange found in certain plant species. The genetic evidence indicates that in reciprocal translocations *A* and *C*, chromosomes II and III broke at the points of spindle fiber attachments and the two left limbs became attached to each other as did the two right limbs. If we represent chromosome II as *Aa* and chromosome III as *Bb* then the interchange chromosomes are *AB* and *ab*. As in *Zea*, six types of chromosome combinations are produced. When alternate chromosomes pass to opposite poles in the heterozygous flies the gametes will contain *Aa-Bb* and *AB-ab*. Four other types are also produced in smaller numbers and give inviable zygotes unless combined with a complementary class. These types are *Aa-AB*, *ab-bB*, *Aa-ab*, and *AB-Bb*. The fact that such irregular gametes will function in *Drosophila*, but not in *Zea* and *Oenothera*, is attributed by the authors to the more complex gametic development in plants.

In translocation *B*, chromosome III broke at the point of the fiber attachment and II somewhat to the left of the fiber attachment. Heterozygous flies produce only two classes of gametes *Ab-Aa* and *bB-Ba*, due to the fact that the chromosome containing more than half of chromosome II and the normal II always pass to opposite poles. Since both classes of gametes are irregular the degree of sterility should be even greater than in translocations *A* and *C*, which is the case.

In 1927 BELLING suggested that segmental interchange between non-homologous chromosomes would account for the chromosome rings found in *Oenothera* by GATES, CLELAND and others. DARLINGTON in 1929 published, in the same journal, a more complete discussion of this hypothesis, without however, referring to BELLING's earlier work. DARLINGTON assumes that a 14-chromosome ring would consist of two complexes; one with the normal haploid complement, and the other consisting of 7 segmental interchange chromosomes. Segmental interchange is assumed to be advantageous in preserving the hybridity of a plant. The larger rings are built up two chromosomes at a time so that any advantage of heterozygosity is increased until a complete ring of chromosomes is formed. Such a ring combined with a balanced lethal system has established two linkage groups which maintain a constant heterozygous condition of the plant.

CLELAND and BLAKESLEE (1930) have applied the segmental interchange hypothesis to the *Oenothera* problem in considerable detail and have shown that predictions made on this hypothesis are verified by the cytological analysis of hybrids. According to their analysis segmental interchange chromosomes are found in both complexes of species with large rings. For instance *O. Lamarkiana* contains two complexes, *velans* which may be represented as *Aa-Bb-Cc-Dd-Ee-Ff-Gg*, and *gaudens* which will be *Aa-BG-Cb-Dg-Ee-Fc-df*. In each complex there are at least two segmental interchange chromosomes and in *gaudens* the two left ends of non-homologous chromosomes are combined (*BG*) as are the two right ends (*df*). In fact such interchange chromosomes seem to be essential for the presence of interchange chromosomes in both complexes. A union of the two *Lamarkiana* complexes results in one pair of chromosomes, *Aa-Aa*, and a ring of 12 non-homologous chromosomes.

In both *Oenothera* and *Rhoeo* the large circles breed true because of the balanced lethal system which eliminates the homozygous combination of each of the two complexes so that progeny with only pairs of chromosomes are not produced. Since translocations are usually lethal when homozygous, in *Drosophila*, it seems probable that the balanced lethals are due to sectional deficiencies in one or both complexes, as DARLINGTON (1929) has suggested. STURTEVANT and DOBZHANSKY have shown that segmental interchange in *Drosophila* does not cause lethals due to the new arrangement of parts, because flies which receive translocation A from one parent and C from the other parent are fully viable.

In cases of segmental interchange where only the heterozygous types are viable there must be lethals which eliminate both complexes in the homozygous condition. If lethals are due to deficiencies resulting from segmental interchange then both complexes must contain at least one segmental interchange chromosome. The constitution of the complexes of ring formation in *Oenothera* as indicated by the analysis of CLELAND and BLAKESLEE seems to be in accord with this hypothesis while the type of exchange postulated by DARLINGTON is not.

On the hypothesis that both complexes must contain interchange chromosomes in species which breed true for ring formation, the chromosome constitution of *Rhoeo* is indicated in Diagram 1. The approximate relative lengths of the chromosomes are shown in this diagram as are the positions of the spindle fibers. When alternate chromosomes in the ring pass to opposite poles each gametic complex

Diagram 1. The two complexes of *Rhoeo* chromosomes are shown in their relative proportions, and the assumed constitution of each end of each chromosome is indicated by letters. A capital letter indicates the left end of a chromosome and a small letter the right end. No two chromosomes are homologous.

will contain six chromosomes. One of these may be designated as *Aa-Bb-Cc-DE-ed-Ff* and the other as *aB-bC-cD-Ee-dF-FA*. When these gametic chromosomes are combined they will form a ring of 12 chromosomes: *Aa-aB-Bb-bC-Cc-cD-DE-Ee-ed-dF-Ff-fA*. Since no forms of *Rhoeo* are known where the chromosomes unite as bivalents it is of course impossible to determine which chromosomes are the original ones and which are the interchange chromosomes. It is necessary that at least 6 of the 12 chromosomes have been produced by segmental interchange, but it is possible that all of them are segmental interchange chromosomes. The designation of the different chromosome segments is of course more or less arbitrary but they do show the relations of the different ends of the 12 chromosomes.

With the information now available it should be possible to postulate some of the probable causes of segmental interchange. It is clear that segmental interchange may be due to breaks at the point of the spindle fiber attachment or at other places on the chromosome. The two right ends of non-homologous chromosomes may be combined as are the two left ends, or the right end of one may unite with the left end of another. Irregular combinations of chromosomes which are deficient for an entire segment apparently do not survive in plants. In true-breeding forms the rings of chromosomes are maintained by a system of balanced lethals, due perhaps to sectional deficiencies.

Segmental interchange between non-homologous chromosomes is, in some respects, very similar to simple translocation and can be regarded as a reciprocal translocation. It seems quite possible that the same type of breaks will often produce either a single translocation or a reciprocal translocation. MORGAN, BRIDGES, and STURTEVANT (1925) have suggested that translocations may be caused by the interlocking of non-homologous chromo-

somes during the progress of synapsis as described by GELEI. If the interlocking is not readily released at later stages then any strain may break the chromatids at the point of contact, presumably at subterminal chiasmata, so that a section from one chromosome might become permanently attracted to another non-homologous chromosome.

An example of such a hypothetical translocation is shown in Diagrams 2, 3, and 4. The bivalent chromosomes become interlocked so that a sub-terminal chiasma of $Aa-Aa$ meets a nearly terminal chiasma of $Bb-Bb$.

Diagrams 2, 3 and 4. The origin of translocation or segmental interchange near the ends of non-homologous chromosomes due to breaks at chiasmata is indicated in these figures.

The two inner chromatids of each bivalent will come in contact if the chromosomes tend to separate. As a result breaks may occur at the point of contact so that a section of segment a will become attached to segment b . The chiasma at bb would be easily separated if completely terminal so that we have

assumed that it is somewhat sub-terminal and would undergo some stress before unravelling. Thus when b and a break and recombine at the point of contact a small terminal section of b might be lost and chromosome Bba would carry a sectional deficiency. Such a chromosome might be lethal in the homozygous condition as is the case in most of the translocations found in *Drosophila*.

If the interchange were reciprocal then the two interchange chromosomes would be Bba and Aab , or in respect to their ends, Ba and Ab . These segmental interchange chromosomes combined with the normal chromosomes would produce a ring of four chromosomes at meiosis, $Aa-aB-Bb-bA$. The interchange segments would probably have to be longer than indicated in the diagram to insure pairing which is essential for ring formation.

The above suggestions seem plausible for certain types of translocations and some cases of segmental interchange. Where translocations occur at or very near the fiber constrictions, however, it seems more probable that the entire chromonema breaks at the point of contact when two non-homologous chromosomes are interlocked. In such

breaks it is also possible that deficiencies would occur but perhaps not as frequently as breaks at chiasmata. In *Oenothera* the interchange seems to occur at or near the fiber constriction and chromosome rings are commonly found in this genus. It seems significant that in *Oenothera* interlocking of non-homologous chromosomes frequently occurs. CLELAND (1922) has pictured and described as many as three bivalents linked with a ring of four chromosomes, and ILLICK (1929) has recently described a case in an F_2 segregate of a species cross in *Oenothera* where all 7 bivalents are apparently linked together. It is perhaps also significant that in *Oenothera*, *Datura* and *Zea*, where segmental interchange is known to occur, the homologous chromosomes at diakinesis are usually attached only at the two ends so that each bivalent is ring shaped. If two such bivalents become interlocked at synapsis and occasionally break at or near the spindle fiber attachment it would be possible to account for the types of interchange found in *Oenothera*, *Datura*, *Rhoeo* and perhaps those of *Zea*.

An example of chromosome interlocking is shown in Diagram 5. Bivalents *Aa-Aa* and *Bb-Bb* have interlocked and are in contact at the spindle fiber constrictions. A break in the chromonemata at this point and a reunion of non-homologous segment would result in either of two types of segmental interchange chromosomes, *AB* and *ab* where the two right ends of non-homologous chromosomes are united as are the two left ends, or *Ab* and *aB* where the left end of *Aa* has united with the right end of *Bb*. (Diagrams 6 and 7). Thus both the *Drosophila* and the *Zea* types of interchange would be accounted for. These segmental interchange chromosomes combined with the two normal chromosomes would form a ring of four in either case. It must be assumed that such interchange could also take place at other points along the chromosomes as is the case in *Zea*. However, in the *Zea* type of interchange both breaks must occur on the same side of the spindle fiber in respect to the proximal end of the chromosome while in the *Drosophila* type the interchange must occur on opposite sides of the spindle fiber attachments, although of course the designation of right and left ends of the second and third chromosomes in *Drosophila* is purely arbitrary and may not be correlated with any polarization of the chromosomes.

If in interchange a sectional deficiency occurred the segmental interchange chromosomes would be lethal when homozygous. But it seems quite probable that interchange may often occur without any lethal factors as seems to be the case in *Datura*. When the chromo-

Diagrams 5, 6 and 7. Interlocked non-homologous chromosomes in contact at or near the spindle fiber constrictions. If these chromosomes break at the point of contact and non-homologous segments reunite two types of segmental interchange chromosomes will be produced, which when combined with normal chromosomes will produce a ring of four non-homologous chromosomes. Interchange is assumed to occur at an early stage, but for convenience a later stage is represented in the diagrams. Diagrams 8 and 9. The chromosome ring is built up by the interlocking of a third bivalent in a ring of 4 chromosomes. The break would produce a ring of 6 non-homologous chromosomes. One of the complexes contains only normal chromosomes so that such a plant should produce both ring and bivalent types. Diagrams 10 and 11. A method of ring formation where each complex contains at least one segmental interchange chromosomes. If lethals are caused by segmental interchange each chromosome complex must contain at least one interchange chromosome in types which breed true for rings.

some ring is complete the presence of a single lethal in each complex would be sufficient to insure the elimination of all bivalents.

The chromosome ring might be built up by the interlocking of additional bivalents and the subsequent interlocking of larger rings or the number of chromosomes in the ring might be increased by 2 pairs of chromosomes at a time. If a third bivalent becomes interlocked with a ring of four a ring of six might be formed as shown in Diagrams 8 and 9. Such a chain of chromosomes would have at least one of the complexes without lethals, assuming lethals to be due to deficiencies originating with segmental interchange, so that both rings and bivalents should be produced from such a ring. If the interchange occurs as indicated in Diagrams 10 and 11 both complexes might carry lethals and only plants with a ring of 6 chromosomes would be viable.

In order to have a ring with both complexes containing at least on interchange chromosome it seems to be necessary to have both types of interchange; the union of two right or left ends of non-homologous chromosomes and the union of the left end of one chromosome with the right end of the other.

This theory of segmental interchange has several weak points. If there is enough strain on the united bivalents to cause a break it would seem improbable that the non-homologous segments would remain attached. Segmental interchange described in *Drosophila* has been produced in the mature sperm by the action of X-rays. In *Oenothera*, however, there is evidence that segmental interchange most occur, in some cases at least, between non-homologous chromosome segments in opposite complexes of the ring. Apparently segmental interchange can occur in several ways.

It might also seem that when ring formation is complete no further segmental interchange could occur, but DARLINGTON has presented a plausible and logical explanation of the origin of Halbmutanten, on the basis of further segmental interchange in the ring of twelve chromosomes in *Oenothera Lamarkiana*. According to DARLINGTON, a further exchange between non-corresponding segments of opposite complexes will result in two new types of gametes, and either of these gametes combined with either *velans* or *gaudens* will produce the Halbmutanten with a ring of 6 and 4 pairs. Actually such new complexes will not always give a ring of 6 and 4 pairs, but will give also a ring of 8 and 3 pairs in half of the new combinations. However, this fact does not necessarily discredit his theory since the combinations with a ring of 8 and 3 pairs may not be viable.

The arrangement of the chromosomes in a rather definite sequence in *Rhoeo* proves conclusively that their orientation is not merely a matter of chance. The heterobrachial chromosomes are invariably united by their proximal ends and the "pairs" of heterobrachial chromosomes are always united by a more or less isobrachial chromosome. One of these heterobrachial chromosomes *Cc* is somewhat longer than the others and it is always attached to the shorter arm of the connecting chromosome *Cb*. The order of the chromosomes proves conclusively the homologous chromosomes are not associated end-to-end in the ring. In fact the morphology of the chromosomes and their behavior at meiosis indicates that there are no homologous chromosomes in *Rhoeo*.

When two or three chains of chromosomes are found at "diakinesis" it is found that segmentation of the ring has usually occurred between the longer chromosome segment rather than between the short segments (Figs. 4, 5 and 6). Segmentation might be expected to occur most frequently between the short chromosome segments if interchange has occurred at the point of spindle fiber attachment, because there would be less opportunity for the formation of chiasmata and those formed would be more easily terminalized. If segmental interchange occurs near the middle of the heterobrachial chromosomes it seems improbable that a chiasma would be formed between the point of interchange and the fiber attachment because terminalization would separate sister chromatids at the point of fiber attachment. The genetic evidence in *Drosophila* indicates that sister chromatids are always associated at the point of spindle fiber attachment. The greater frequency of breaks in the ring between the longer chromosome segments might be due to greater frequency of crossing over in these segments. If these segments are associated by a single chiasma a break in the chiasma would not only result in a crossover but would also result in a temporary break in the chromosome-ring.

Rhoeo discolor is a monotypic genus and no forms with bivalents have been discovered. All of the plants have rings of chains of 12 chromosomes. Some of the related genera have 6 pairs of chromosomes and there is some tendency for ring formation to occur in other genera of the same family. If the present forms of *Rhoeo* originated from diploid forms by segmental interchange it seems strange that only the ring types have survived, since they are relatively sterile.

About 80 to 90 percent of the pollen grains of *Rhoeo* are obviously imperfect. Much of this sterility can undoubtedly be attributed to

non-disjunction which occurs in about half of the first meiotic divisions. But there must be additional factors to account for the high degree of sterility found.

Several cases of asynapsis, presumably caused by low temperatures, have previously been reported. BELLING has suggested that chromosome pairing in tropical or subtropical plants might be more easily disturbed by cold than in plants of the temperate zone. *Rhoeo* is not only tropical but the chromosomes are attached only at their ends so that asynapsis might be expected to occur frequently in this genus. As was suggested in an earlier paper (SAX 1930) asynapsis may be due to a rapid rate of chromatid development in relation to chromosome pairing. Such differential development may apparently be caused by genetic factors or by environmental conditions.

The writer is indebted to Dr. E. M. EAST and Dr. R. E. CLELAND for valuable suggestions.

Conclusions

As previous investigators have found, *Rhoeo discolor* has 12 somatic chromosomes which form either a ring, or one to three chains at meiosis. In about half of the reduction divisions alternate chromosomes pass to opposite poles, but various types of non-disjunction are frequently found.

When the meiotic chromosomes of *Rhoeo* are properly fixed and stained it is possible to follow the sequence of 8 of the 12 chromosomes in the ring. Two heterobrachial chromosomes are followed by long approximately isobrachial chromosome which is attached to another "pair" of heterobrachial chromosomes, followed by a short isobrachial chromosome and a less distinctly heterobrachial "pair." The heterobrachial chromosomes are invariably attached at their proximal ends. These chromosomes are always found in the same order in the ring. In every respect they conform to the requirements of BELLING's segmental interchange hypothesis.

It is suggested that segmental interchange may be caused by interlocking of non-homologous bivalent chromosomes during synapsis with subsequent breaks at the point of contact and a reunion of non-homologous segments.

The origin of balanced lethals in species which breed true for chromosome rings is attributed to occasional deficiencies occurring with segmental interchange.

When *Rhoeo* plants are subjected to low temperature asynapsis frequently occurs so that twelve univalent chromosomes are found at the first meiotic division. These chromosomes divide equationally at both "reduction" divisions.

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Literature Cited

- Belling, J. 1927 The attachments of chromosomes at the reduction division in flowering plants. Journ. Gen. 18: 177-205.
- Blakeslee, A. F. and R. E. Cleland, 1930 Circle formation in *Datura* and *Oenothera*. Proc. Nat. Acad. Sci. 16: 177-183.
- Burnham, C. R. 1930 Genetical and cytological studies of semisterility and related phenomena in Maize. Proc. Nat. Acad. Sci. 16: 269-277.
- Cleland, R. E. and A. F. Blakeslee, 1930 Interaction between complexes as evidence for segmental interchange in *Oenothera*. Proc. Nat. Acad. Sci. 16: 183-189.
- 1922 The reduction divisions in the pollen mother cells of *Oenothera franciscana*. Amer. Journ. Bot. 9: 391-413.
- Cooper, D. C. and R. A. Brink, 1931 Cytological evidence for segmental interchange between non-homologous chromosomes in Maize. Proc. Nat. Acad. Sci. 17: 334-338.
- Darlington, C. D. 1929 Chromosome behavior and structural hybridity in the *Tradescantiae*. Jour. Gen. 21: 207-286.
- 1929 Ring formation in *Oenothera* and other genera. Jour. Gen. 20: 245-363.
- Hoar, C. S. 1931 Meiosis in *Hypericum punctatum* Lam. Science 73: 528-529.
- Illick, J. T. 1929 A cytological study of meiosis in the pollen mother cells of some *Oenotheras*. Genetics 14: 591-633.
- Kato, K. 1930 Cytological studies of pollen mother cells of *Rhoeo discolor* Hance, with special reference to the question of the mode of syndesis. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. 5: 139-161.
- McClintock, B. 1930 A cytological demonstration of the location of an interchange between two non-homologous chromosomes of *Zea Mays*. Proc. Nat. Acad. Sci. 16: 791-796.
- Morgan, T. H., C. B. Bridges and A. H. Sturtevant, 1925 The genetics of *Drosophila*. Biblio. Gen. 2: 1-262.
- Sax, Karl 1930 Chromosome structure and the mechanism of crossing over. Jour. Arnold Arb. 11: 193-220.
- Sturtevant, A. H. and T. Dobzhansky, 1930 Reciprocal translocations in *Drosophila* and their bearing on *Oenothera* cytology and genetics. Proc. Nat. Acad. Sci. 16: 533-536.

Mr. W. C. BARRETT has recently found that the chromosomes form a ring or chain at the first meiotic division in the megaspore mother cell of *Rhoeo discolor*.