[4] Blakeslee, A. F., and Cleland, R. E., "Segmental Interchange, a Possible Basis of Chromosomal Attachments in Oenothera."

[5] Cleland, R. E., *Amer. J. Bot.*, **9**, 391–413 (1922).

[6] Renner, O., *Berichte Deutsch. Bot. Gesell.*, **34**, 858–869 (1917); *Zeitschr. indukt. Abst.-u. Vererb.*, **18**, 121–294 (1917); *Flora*, **11**, 641–667 (1918); *Bibliotheca Genetica*, 9 (1925).

[7] Belling, J., and Blakeslee, A. F., these Proceedings, **12**, 7–11 (1926).

[8] Blakeslee, A. F., and Cartledge, J. L., *Mem. Hort. Soc. N. Y.*, **3**, 305–312 (1927).

[9] Håkansson, A., *Hereditas*, **11**, 129–181 (1928).

[10] Darlington, C. D., *J. Genet.*, **20**, 345–363 (1929).

[11] DeVries, H., *Genet.*, **8**, 233–238 (1923).

INTERACTION BETWEEN COMPLEXES AS EVIDENCE FOR SEGMENTAL INTERCHANGE IN OENOTHERA[1]

By Ralph E. Cleland and Albert F. Blakeslee

Goucher College, Baltimore, Md., and Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y.

Communicated February 13, 1930

In the immediately preceding paper, the formation of circles of 4 or more attached chromosomes during diakinesis has been shown to be explainable on the hypothesis of segmental interchange, and certain relations of this hypothesis to phenomena in Oenothera have been pointed out.

The real test of an hypothesis lies in the success of predictions based upon it. We shall show in the present paper that it is possible successfully to predict on the basis of this hypothesis what the chromosome configuration ought to be in certain complex-combinations. The method is to consider complexes in groups of 3, each complex corresponding to a corner of a triangle, while the chromosome configuration which results when two complexes of the triangle are combined is represented by the side of the triangle connecting the two complexes in question. When the configurations are known for two sides of the triangle, it will be found that in some cases at least the configurations possible to the third side are few in number on the basis of segmental interchange. The configuration representing the third side can therefore be predicted, at least to the extent of reducing the possibilities to a few of the 15 configurations theoretically obtainable in the genus. We have first considered triangles, all three sides of which are known, in order to check the possibilities of this method. Finding that the actual configurations are in each case one of the theoretically predictable possibilities (in some cases the only possibility), we have ventured to make predictions in respect to complex-combinations whose configurations are as yet unknown.

For example, take the triangle *excellens*–^{h}*Hookeri*–*flavens*.[2]

Excellens.^{h}*Hookeri* has a circle of 4, and 5 pairs[3] and ^{h}*Hookeri.flavens* has also a circle of 4, and 5 pairs.[4] We shall consider the chromosome configuration of *excellens.flavens* for the moment as unknown. On the basis of segmental interchange, it is theoretically possible for *excellens. flavens* to have one of 4 configurations only. These are listed in paragraphs (*a*) to (*d*).

(*a*) *Seven pairs.*—*Excellens* and *flavens* both differ from ^{h}*Hookeri* by a single interchange. They might, therefore, have identical arrangement of ends, in which case, however, they would give, when combined with each other, 7 pairs. This theoretical possibility is not actually possible, however, for if *excellens* and *flavens* have the same end arrangement they must give the same configurations when combined with a given third complex. This they do not do when combined with *gaudens* and *velans*.

(*b*) *Circle of 4, 5 pairs.*—The interchanges giving rise to both *excellens* and *flavens* might have involved the same chromosomes, but different ends have been exchanged in the two cases. *Excellens* and *flavens* would then yield with each other a circle of 4, and 5 pairs.

Let ^{h}*Hookeri* = 1·2, 3·4, 5·6, 7·8, 9·10, 11·12, 13·14
Let *flavens* = 1·4, 3·2, 5·6, 7·8, 9·10, 11·12, 13·14
Let *excellens* = 1·3, 2·4, 5·6, 7·8, 9·10, 11·12, 13·14

This possibility may be tested by seeing whether the complexes of the triangle as arranged will give the proper configurations when combined with still other complexes. Using *velans* in this case as a tester, it is found that when *excellens* and *flavens* are so arranged as to give with each other a circle of 4, they cannot both give the proper configuration with *velans*. *Velans* gives with *excellens* a circle of 6 and 4 pairs;[3] and with *flavens* 2 circles of 4 and 3 pairs.[5] But no complex that will give 2 circles of 4 with *flavens* can give a circle of 6 with *excellens* when *flavens* and *excellens* bear the relation they do in the above formulæ. Possibility (*b*) must therefore be eliminated.

(*c*) *Circle of 6, 4 pairs.*—One of the 2 chromosomes involved in the interchange resulting in the *flavens* end arrangement might also have been involved in the formation of the *excellens* arrangement. *Excellens.flavens* should then have a circle of 6 and 4 pairs.

Let ^{h}*Hookeri* = 1·2, 3·4, 5·6, 7·8, 9·10, 11·12, 13·14
Let *flavens* = 1·4, 3·2, 5·6, 7·8, 9·10, 11·12, 13·14
Let *excellens* = 1·2, 3·6, 5·4, 7·8, 9·10, 11·12, 13·14

With the data at hand, we have not been able as yet to eliminate this possibility. Thus, using *velans*, *punctulans*[2] and *gaudens* as testers (these being the only 3 complexes whose configurations are known with all 3 of the triangle as well as with each other) it is possible to construct formulæ for all 6 complexes such that each will give the correct chromosome configuration with each of the others, as for instance:

Let *velans*	= 1·2,	3·4,	5·8,	7·6,	9·10,	11·12,	13·14
Let *punctulans*	= 1·4,	3·8,	5·6,	7·10,	9·12,	11·2,	13·14
Let *gaudens*	= 1·2,	4·6,	5·11,	7·8,	9·14,	10·12,	13·3

(*d*) *Two circles of 4, 3 pairs.—Excellens* and *flavens* might differ from *hHookeri* by an interchange involving entirely different pairs of chromosomes. They would then give when combined with each other 2 circles of 4 and 3 pairs.

Let *hHookeri*	= 1·2,	3·4,	5·6,	7·8,	9·10,	11·12,	13·14
Let *flavens*	= 1·4,	3·2,	5.6,	7·8,	9·10,	11·12,	13·14
Let *excellens*	= 1·2,	3·4,	5·8,	7·6,	9·10,	11·12,	13·14

With the triangle arranged in this way, the following formulæ for *velans*, *punctulans* and *gaudens* will meet every demand as far as known:

Let *velans*	= 1·2,	3·4,	5·10,	7·8,	9·6,	11·12,	13·14
Let *punctulans*	= 1·4,	3·10,	5·6,	7·12,	9·8,	11·2,	13·14
Let *gaudens*	= 1·2,	3·13,	5·4,	7·14,	9·10,	11·6,	8·12

This is actually a possibility therefore as far as we can tell from available cytological data.

Our conclusion, therefore, in regard to *excellens.flavens* is that this complex-combination must have on the basis of segmental interchange either a circle of 6 and 4 pairs, or else 2 circles of 4 and 3 pairs. It has, as a matter of fact, the latter configuration.[3]

The other sides of this triangle may be similarly tested. Considering *excellens.hHookeri* as the unknown, the following are the theoretical possibilities for this combination, so far as this triangle is concerned—circle of 4 and 5 pairs; 2 circles of 4 and 3 pairs; 3 circles of 4 and 1 pair; circle of 4, circle of 6 and 2 pairs; circle of 8 and 3 pairs. Using *velans*, *gaudens* and *punctulans* as testers, the only configuration which survives the test is circle of 4 and 5 pairs. That is, when the complexes of the triangle are so arranged that *excellens.hHookeri* has any of the theoretical possibilities other than a circle of 4, it is impossible to form *velans*, *gaudens* and *punctulans* which will give the correct chromosome configuration with each of the triangle and with each other. On the basis of segmental interchange, therefore, *excellens.hHookeri* must have a circle of 4 and 5 pairs, nothing else. As a matter of fact, it has this configuration.[3]

The third side of the triangle, *hHookeri.flavens*, in the same way may have theoretically, as far as this triangle is concerned, a circle of 4, 5 pairs; 2 circles of 4, 3 pairs; 3 circles of 4, 1 pair; circle of 4, circle of 6, 2 pairs; and a circle of 8, 3 pairs. Two possibilities remain after *velans*, *gaudens* and *punctulans* have been used as testers—namely, circle of 4, 5 pairs; or circle of 4, circle of 6, 2 pairs. Of these, the former is the actual configuration.[4]

In like manner, other complex-combinations, whose configurations are already known, have been tested and in each case the actual configuration is one of the possibilities under segmental interchange. Thus, *velans.stringens* must have 3 circles of 4, 1 pair; or circle of 4, circle of 6, 2 pairs. It has the latter.[5] *^{h}Hookeri.velans* must have a circle of 4, 5 pairs; or a circle of 4, circle of 6, 2 pairs. It has the former.[3]

Such success in making predictions upon the basis of segmental interchange has led us to make predictions in regard to some complex-combinations whose configurations are as yet unknown. Thus, we venture to predict that *^{h}Hookeri.stringens* will be found to have 2 circles of 4 and 3 pairs; that *excellens.stringens* will be found to have 3 circles of 4 and 1 pair, or a circle of 4, circle of 6 and 2 pairs; and that *acuens.flavens* will be found to have 2 circles of 4 and 3 pairs, or a circle of 8 and 3 pairs.[6]

It will be noted that in only 2 of the above 8 examples, has it been possible to reduce the possibilities to a single one. In 6 cases we have had to content ourselves with predicting that the configuration will be one of two possibilities. This is as far as we can go, using only our present knowledge of chromosome configurations as they have been determined in various complex-combinations, and applying these data according to the theory of segmental interchange. There exists, however, a further check which in several of the above cases eliminates one of the two possibilities and makes it possible to reduce the theoretically predictable configurations to one. We are indebted to Dr. A. H. Sturtevant for the suggestion that a study of linkage relations in Oenothera will in some cases lead to eliminations.[7]

The following facts may be applied as tests to the possibilities which have survived in the above examples: Inasmuch as *R* segregates independently of the rest of the complex and of the lethals in *velans.gaudens* (circle of 12), it probably resides in the single independent pair of chromosomes. But on the basis of segmental interchange, chromosomes must have the same end arrangement in order to pair. *R* also segregates independently in *^{h}Hookeri.rubens* (circle of 10),[8] and since *rubens* and *gaudens* are practically identical, presumably also in *^{h}Hookeri.gaudens* (circle of 10). *^{h}Hookeri* and *velans*, therefore, as well as *rubens* and *gaudens* must have the *R* chromosome in common in respect to end arrangement.

Since *R* and *P* segregate independently of each other and of the rest of the complex in *rigens.velans* or *rigens.^{h}Hookeri*, they lie in different and independent chromosomes in *velans* and *^{h}Hookeri*, and these complexes have identical end arrangement in respect to the *P* chromosome, as well as to that containing *R*. But *R* and *P* are linked in *velans.flavens* (2 circles of 4). They must lie, therefore in a single chromosome group in this combination, that is, in one of the circles of 4. A circle of 4 is obtained on the basis of segmental interchange when two chromosomes which have suffered a simple exchange of ends are brought into association with corresponding chromosomes between which no exchange has occurred. Therefore the *R* and *P* chromosomes of *flavens* differ from the corresponding *velans* and ^{h}*Hookeri* chromosomes by a single interchange. In *flavens. stringens P* segregates independently of the rest of the complex.[9] It must therefore lie in a free pair of chromosomes. It follows that *flavens* and *stringens* have the *P* chromosome in common in respect to end arrangement.

Fr (pollen fertility factor) segregates independently in *albicans.stringens* (circle of 12) and *flavens.stringens* (circle of 4),[9] so that *albicans, flavens* and *stringens* must be alike in respect to end arrangement of the *Fr* chromosome. But *albicans,* while having the *Fr* chromosome in common with both *flavens* and *stringens,* has none in common with ^{h}*Hookeri* (*albicans.* ^{h}*Hookeri* has circle of 14). The only one it can have in common with *flavens* is one in respect to which *flavens* differs from ^{h}*Hookeri,* that is, in the *R* or *P* chromosome, or in the other *flavens* chromosome in the circle formed with ^{h}*Hookeri,* if *R* and *P* are in the same *flavens* chromosome. (*flavens.*^{h}*Hookeri* has a circle of 4, 5 pairs). *Fr* resides therefore in one of these. But *Fr* and *P* segregate independently of each other in *flavens. stringens,* so *Fr* and *P* lie in different and independent chromosomes in *flavens* and *stringens,* and *stringens* has not one but both of the chromosomes in common with *flavens* in respect to which *flavens* differs from ^{h}*Hookeri.* Since it is not possible to tell with certainty which of these two *flavens* chromosomes has *R* and which has *P* (that will depend upon the method of interchange) one cannot be certain which of these chromosomes is common to *albicans* and *stringens.* Furthermore, whether *Fr* lies in the same chromosome as *R* in *flavens, albicans* and *stringens* will depend upon the loci of *R* and *P.* (*a*) If both or neither of these two genes are located in the segments which have interchanged, then *R* and *P* will lie in different chromosomes in *flavens, Fr* will lie in the *R* chromosome, and it will be the *R* chromosome which *flavens* and *albicans* have in common. (*b*) If, however, one only of these two genes is in the segments which have interchanged, then both *P* and *R* will lie in the same chromosome in *flavens.* The chromosome common to *flavens, albicans* and *stringens* then would contain neither *R* nor *P,* and *Fr* would lie in the chromosome lacking both *R* and *P.* It may be possible to determine genetically which is the correct alternative. Meanwhile, *albicans* may be said to have in common with *flavens* one of the 2 chromosomes in respect to which the latter differs from ^{h}*Hookeri,* and *stringens* has both of these chromosomes in common with *flavens.*

Using these facts as an added check on the predictions obtained above on the basis of purely cytological data, further eliminations must be made in certain cases, owing to the fact that the complexes as they must be arranged in order to give the predicted configurations do not have the proper chromosomes in common as called for by the genetic data. Thus,

^{h}*Hookeri.flavens* cannot have a circle of 4, circle of 6 and 2 pairs; but only a circle of 4 and 5 pairs (which it actually has). *Velans.stringens* cannot have 3 circles of 4 and 1 pair, but only a circle of 4, circle of 6 and 2 pairs (which it actually has). ^{h}*Hookeri. velans* cannot have circle of 4, circle of 6 and 2 pairs, but only a circle of 4 and 5 pairs (which it actually has).[3]

We may illustrate with ^{h}*Hookeri.velans*. If this combination has a circle of 4, circle of 6 and 2 pairs, the formulæ for the complexes of the triangle ^{h}*Hookeri–flavens–velans* would be as follows:

Let ^{h}*Hookeri*	= 1·2,	3·4,	5·6,	7·8,	9·10,	11·12,	13·14
Let *flavens*	= 1·4,	3·2,	5·6,	7·8,	9·10,	11·12,	13·14
Let *velans*	= 1·4,	3·6,	5·2,	7·10,	9·8,	11·12,	13·14

According to genetic data, *velans* must have in common with ^{h}*Hookeri* the 2 chromosomes in respect to which ^{h}*Hookeri* differs from *flavens* (the *R* and *P* chromosomes). No arrangement of *velans* which will give 2 circles of 4 with *flavens* and a circle of 4, circle of 6 with ^{h}*Hookeri*, however, can satisfy these conditions. Consequently, ^{h}*Hookeri.velans* cannot have a circle of 4, circle of 6 and 2 pairs. It can, however, have a circle of 4 and 5 pairs.

The end result of this attempt at prediction, therefore, is that in the case of 5 hybrid combinations definite predictions have been made as to the chromosome configuration (*excellens.*^{h}*Hookeri*, ^{h}*Hookeri.flavens*, *velans.stringens*, ^{h}*Hookeri.velans*, ^{h}*Hookeri.stringens*), while less definite predictions have been made in 3 other cases (*excellens.flavens*, *excellens. stringens*, *acuens.flavens*). Of the 5 in which definite predictions have been made, 3 were already known cytologically when the predictions were worked out, and the predictions agreed with the facts, one has since been ascertained to be in line with the prediction (^{h}*Hookeri.velans*) and one still remains to be determined (^{h}*Hookeri.stringens*).

A list of hypothetical formulæ for several complexes which meet all cytological demands on the basis of segmental interchange, (i.e., each gives the correct chromosome configuration with each of the others) and which meet also all genetical demands as far as we have been able to determine them is given herewith.

Let ^{h}*Hookeri* = 1·2, 3·4, 5·6, 7·8, 9·10, 11·12, 13·14.

Let 1·2 contain *R*, let 3·4 contain *P*, let 5·6 contain *Sp, let.* Then *flavens* must have 1·4, 3·2 or 1·3, 4·2, the rest of the complex being like ^{h}*Hookeri.*

Let *flavens* = 1·4, 3·2, 5·6, 7·8, 9·10, 11·12, 13·14.

Then *velans* must have 1·2, 3·4 and differ from ^{h}*Hookeri* by one other interchange. Selecting the third and fourth chrcmosomes arbitrarily (neither ^{h}*Hookeri* nor *velans* carries *sp* nor *let*, hence the correctness of this choice is not known).

Let *velans* = 1·2, 3·4, 5·8, 7·6, 9·10, 11·12, 13·14.

Excellens must have 1·2, 3·4 in order to give a circle of 4 with *ʰHookeri*, and 2 circles of 4 with *flavens*. The interchange must involve 5·6 or 7·8 (not both) in order to give a circle of 6 with *velans*. Choosing arbitrarily 7·8 and 9·10 for the interchange,

Let *excellens* = 1·2, 3·4, 5·6, 7·10, 9·8, 11·12, 13·14.

Stringens has 1·4, 3·2 like *flavens*. To give 2 circles of 4 with *ʰHookeri*, a circle of 4 with *flavens*, and a circle of 4, circle of 6 with *velans*, an exchange must have occurred between either 5·6 or 7·8, and one of the last 3 chromosomes. Choosing 5·6 and 9·10 arbitrarily,

Let *stringens* = 1·4, 3·2, 5·10, 7·8, 9·6, 11·12, 13·14.

Rubens and *gaudens* have 1·2 like *velans* and 5·6 like *ʰHookeri* (*Sp, sp* segregate independently in *ʰHookeri.rubens*). As nothing is known respecting the position of the genes in the other chromosomes, they may be arranged tentatively in any way to give the correct configurations with the other complexes.

Let *rubens* and *gaudens* = 1·2, 3·14, 5·6, 7·4, 12·10, 11·8, 13·9.

Albicans must have 1·4 or 3·2 like *flavens* and *stringens*. The other chromosomes may be arranged tentatively to give the proper configurations with other complexes.

Let *albicans* = 1·4, 3·5, 6·8, 7·10, 9·12, 11·14, 13·2.

The neatness with which one is able to predict the chromosome configurations in various complex-combinations on the basis of segmental interchange leads to the conclusion that the phenomenon of segmental interchange is probably at the basis of circle formation in Oenothera. A fuller discussion of segmental interchange as applied to Oenothera is soon to appear elsewhere.

[1] Aided by a grant to the first author from the BACHE FUND of the NATIONAL ACADEMY OF SCIENCES.

[2] *Excellens* and *punctulans* are unpublished names applied tentatively by Renner to the egg and pollen complexes, respectively, of the "*biennis* Chicago" of de Vries (*Chicagoensis* of Renner). We are indebted to Professor Renner for permission to use these names.

[3] Unpublished data.

[4] Cleland, R. E., *Zeitschr. indukt. Abst.- u. Vererb.*, 1928, Suppl. Band **1**, 554–567 (1928).

[5] Cleland, R. E., and Oehlkers, Fr., *Am. Nat.*, **63**, 497–510 (1929).

[6] Gerhard assigns to *acuens. flavens* a circle of 4 and 5 pairs (*Zeitschr. Naturwiss.*, **64**, 283–338 (1929)), but inasmuch as his *grandiflora* seems to have a different chromosome configuration from that described by Cleland and Oehlkers, the material used in the two cases is probably not comparable.

[7] In a letter received from Dr. Sturtevant after the above predictions had been made, we learn that he and Dr. S. H. Emerson have together arrived independently at some of the same predictions that we have made.

[8] Renner, O., *Bibliotheca Genetica*, **9** (1925).

[9] Oehlkers, Fr., *Jahrb. wiss. Bot.*, **65**, 403–446 (1926).