Other consequences of the interchanges in *Oenothera*

Pollen sterility

According to Sturtevant's review of Renner's studies, the pollen of O. hookeri, a species with 7II of chromosomes, was practically all well filled and capable of germination. "In all other species roughly half of the grains were small and empty." These aborted grains were present also in lamarckiana in which both complexes were functional in both pollen and eggs. Since chromosome orientation in the rings is upwards of 80 to 90% alternate (zigzag configurations), pollen fertility should be high even in the largest rings. Sterility from crossing over in interstitial segments is expected to be low, partly because few such segments are present in most rings, and even when present, crossing over in segments adjacent to the centromere is presumably low.

Irregular segregations from the rings, 6 chromosomes (n-1) passing to one pole, 8 (n + 1) to the other; or 9-5 disjunctions, and lagging chromosomes also produce some spores which are deficient and are expected to abort. The frequencies of irregular configurations and lagging for races with configurations of various sizes were determined by Cleland and Oehlkers (1930). Their observations on chromosome segregation and extra nuclei are summarized in Table 43.

Table 43. Summary of frequencies of abnormal meiotic behavior in Oenothera races having various chromosome configurations (from observations reported by Cleland and Oehlkers, 1930).

No. of different species or hybrids	Configuration	Total	Metaphase I % irregular zigzag	Chromosome separation as seen at interkinesis	
				Total obs.	% irregular*
6	⊙ 14	905	32.7	1747	14.9
7	⊙ 12	1043	16.5	2187	10.0
5	⊙ 10, ⊙ 4 or ⊙ 10	740	9.9	1201	8.3
2	⊙ 8, ⊙ 6	319	10.9	426	1.9
3	⊙ 6, ⊙ 4	567	1.6	657	1.8
5	2 ⊙ 4 or ⊙ 4	3613	2.3	1061	1.3

* includes extra nuclei

Most of the irregular separations in the last column (Table 43) were 8-6 chromosome segregations. Lagging chromosomes were recorded rarely. Dyads with extra nuclei were rare although one hybrid with a ⊙ 14 had about 4%, one with a ⊙ 12 had about 6% and one with a ⊙ 10 had about 8%. The larger rings showed strikingly higher frequencies of irregularity at metaphase I, especially the races with rings of 14 chromosomes. A considerable portion of these irregularities is expected to result in deficient spores which should abort. The values in the last column appear to account for very little sterility. Irregular disjunctions may balance each other to produce spores which are deficient and yet have the normal chromosome number, for example, in a disjunction of this type

, 3 chromosomes would pass to each pole, but both would be deficient. One segregation of two adjacent chromosomes toward one pole is counterbalanced by a similar segregation to the other pole at another point in the configuration. Hence the

irregularities in the last column may represent only a relatively small portion of those which should result in abortive spores.

Other abnormalities include breakage of the large ring into a chain or into two or more chains. The numbers of these seem to vary for different hybrids showing the same ring size, and even in the same complex-combination resulting from reciprocal hybrids. The differences should be related to relative lengths of interchanged pieces, although the ability of the chromosomes to hold together is affected also by temperature and other environmental factors (Oehlkers, 1935, 1936, Harte 1953). Some of these irregularities might add to the observed sterility also. For example, in those cells having two or more chains, the mechanism for alternate segregation could no longer operate for all the interchanged chromosomes.

Cleland states that the percentage abortion is highly variable, varying from year to year and week to week in the same stock. Counts of the frequencies of shriveled and empty pollen were reported by Cleland (1935a, 1937) for many of the races and hybrids with various chromosomal configurations. These are summarized in Table 44.

Table 44. Summary of observations on the amount of shriveled and empty pollen in races and hybrids with different chromosome configurations (from Cleland 1935a, 1937).

Configurations	Per cent of shriveled and aborted pollen								Total No.	Avg. %
	0-15	16-25	26-35	36-45	46-55	56-65	66-75	76-		
14*	-	1	3	5	4	5	3	1	22	50.7
12	-	-	1	1	1	5	2	3	13	62.7
10	-	-	6	5	2	1	-	1	15	41.3
8	-	1	2	2	1	2	-	-	8	43.2
6	2	3	2	-	1	-	1	1	10	35.1
4	7	2	-	-	-	1	1	-	11	22.2
7 II	7	2	-	-	-	-	2	1	12	9.8**
10,4	-	-	1	-	-	2	-	-	3	49.1
8,4	-	-	1	1	1	1	1	1	6	56.1
6,6	-	1	-	-	-	-	-	-	1	22.9
6,4	-	1	1	1	1	3	-	-	7	44.9
6,4,4	-	-	-	-	1	-	-	-	1	46.8
4,4,4	-	-	-	-	1	-	-	-	1	46.4
4,4	1	1	1	1	1	1	2	-	8	43.5

* = ring of 14, etc.

** = the three high steriles not included.

The average percentages of shriveled pollen shown in Table 44 were roughly 50% for all races with large rings or for those with two or three rings. Of those with a ring of 14 chromosomes two showed 25% of abnormal pollen, one a high value of 75%. Cleland and Hammond (1950) reported pollen abortion counts for four races with a ⊙ 14 as 4.3, 27.8, 14.1 and 43.7%. Those with 7 pairs had pollen abortion values between 5.2 and 21.4% (Table 44). Among those with a ⊙ 4 there was one with only 5% and one with 7%; similar to certain of those with 7 pairs. One hybrid with a ⊙ 4 in which no irregular zigzags were found still showed 65% of aborted pollen. Since abortion shows no close

and three had high values.

relationship to the size of the ring, other factors not as yet analyzed must be involved. The variation caused by environment has been mentioned.

In certain races, the sterility might be caused by a "gene" for pollen abortion which is transmitted through the ♀ but not through the pollen. These have been reported in maize (Rhoades and Rhoades 1939, Singleton and Mangelsdorf 1940, Burnham 1941), and in Datura (Cartledge and Blakeslee 1934). In a plant heterozygous for one of these, the pollen is 50% aborted and seed set is normal. In maize, pachytene analysis revealed no recognizable chromosomal change. Certain of the pollen lethals in Oenothera may be of this type, but only if they were close to an interchange point would they show complete linkage with a certain complex in a balanced lethal arrangement. If far enough away they would show independence with the complexes. In that case different strains of the same race should show differences in sterility. One of the races has been described as differing in pollen sterility in different collections. A study of pollen sterility in races with seven chromosome pairs might furnish needed information.

Origin of new types, also half-mutants

Lamarckiana and several other races of Oenothera throw a class of new types called "half-mutants" by deVries. For example, the half-mutant rubrinervis was reported by deVries (1919) to originate from O. lamarckiana in his garden in a percentage of about 0.1. When selfed it produced a ratio of 2 rubrinervis: 1 fertile and constant deserens: 1 inviable (empty) seeds. Later cytological studies showed that lamarckiana had a ⊙ 12, 1 pair; rubrinervis a ⊙ 6, 4 II; and deserens 7 II (Cleland 1925). The half-mutants from other races usually had a ⊙ 6 and 4 pairs or a ⊙ 8 and 3 pairs, while the pure-breeding derivatives have always had 7 II. A possible explanation is that these half-mutants result from crossing over between a differential segment and its homologue in another chromosome in the ring (Emerson 1936). If one of the original chromosomes is involved in two different interchanges and the breaks are not at exactly the same point, there will be a differential segment as shown in Figure 41.

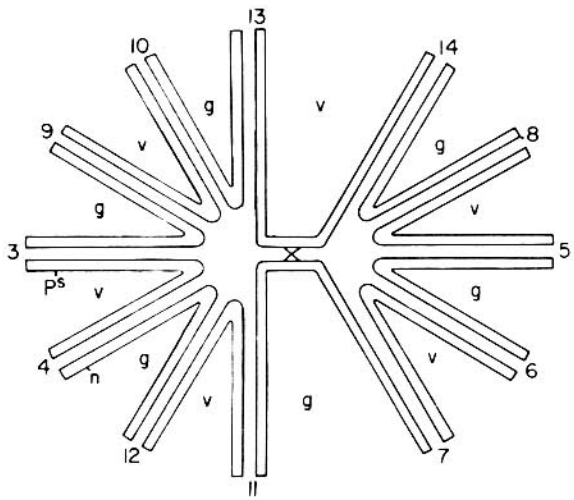

FIG. 41. Diagram illustrating the pachytene configuration expected in the ⊙ 12 in lamarckiana if chromosome 13, 14 has a middle segment which is the homologue of the middle segment of 7, 11. A crossover in that differential segment "results in viable complexes which have some velans and some gaudens chromosomes" (after Emerson, 1935, Fig. 5a, p. 553, Amer. Nat. 69).

Crossing-over in a differential segment such as that shown in Figure 41 will account for the observed recurrence of the same half-mutants, as observed in the progeny of certain races. The crossover shown in Figure 41 might be expected to produce among its self progeny plants with a ring of six or a ring of eight, two kinds of each, if lethals did not preclude their viability. One of these is nn without having had the crossover shown in Figure 38 in arm number 4. This would be viable only if the lethal were not carried on 4.12 but on some other chromosome close to an interchange point in the ring. Since these new types have smaller rings and more pairs, they may form more kinds of viable gametes and produce several new types in their progeny. This may account for the phenomenon of "mass mutation" described by Bartlett (1915). Emerson (1936) in a survey of the information on recurrent half-mutants, concluded that one differential segment (he used the term "interstitial")

is present in each of the pratincola, biennis, and lamarckiana races. Emerson also surveyed the evidence as to which chromosomes do not carry differential segments, pointing out that in any hybrid having a pair plus a large ring, "no large part of the paired chromosome is a differential segment homologous to part of any chromosome in the ring". This does not appear to have a bearing on the presence or absence of differential segments in chromosomes included in the ring. The evidence from chromosome morphology is against the presence of differential segments in most of the interchanges that have survived. There are no marked differences in chromosome morphology or size in the North American Euoenotheras. In Oenothera hookeri (7 II) the chromosome with the satellite has a subterminal centromere, but in the other six the centromere is median or submedian. All have heavily condensed regions adjacent to the centromere and attenuated, lightly staining ends (Wisniewska 1935).

The rarity of races with differential segments simplifies the problem of chromosome analysis, since the determination of homologies is limited to the end-segments.

Crossing over between duplicated segments might lead to new interchanges and exceptional types also. The occurrence of chromosome associations in a haploid is considered as evidence for some duplication of segments (Catcheside 1932).

Extra-chromosome mutants

Trisomic.

Segregation of the chromosomes in the rings is not always regular and may produce viable 8-chromosome gametes, as in Figure 42.

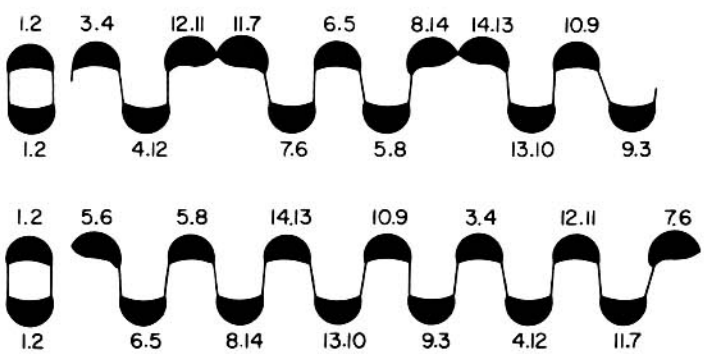

FIG. 42. Upper -- Metaphase orientation of chromosomes in Oe. lamarckiana in which there are two irregularities which will result in an unequal numerical disjunction, but in which the product receiving the larger number has each chromosome represented at least once. (Emerson, 1935, Fig. 6d, p. 556, Amer. Nat. 69).

Lower -- Diagram of regular chromosome disjunction in a trisomic form which has a complete set of lamarckiana chromosomes with 5.6 of gaudens as the extra chromosome. This distribution produces velans + 5.6 and gaudens. The arrangement in which the 5.6 chromosome at the left end is attached on the 7.6 chromosome at the other end of the string will produce velans and gaudens + 5.6 (Emerson, 1935, Fig. 7a, p. 558, Amer. Nat. 69).

In Figure 42, the orientation shows 8 or (n + 1) chromosomes passing to one pole and 6 to the other. Each is a mixture of velans and gaudens chromosomes. The n + 1 combination has the . 11 and . 14 ends in excess, and might be transmitted through the

eggs unless it had picked up the factor that prevents ♀ transmission. The other combination is expected to abort because of the deficiency for the .11 and .14 ends.

In certain cases the 2n + 1 condition might breed nearly true for number, depending on the lethals present and their positions (lower diagram in Fig. 42). Irregular disjunctions occur frequently in the trisomics to give rise to new kinds of aneuploid spores. Sansome and Philp (1939) have calculated that at least 73 different trisomics are possible from O. lamarckiana. Of the original mutants observed by deVries, scintillans, lata, oblonga, and albida were trisomics.

Polyploids

The original Gigas form found by deVries in the progeny of lamarckiana was shown by Miss Lutz in 1907 to be a tetraploid. It had two velans and two gaudens complexes. Renner (1933b) described five fruitful tetraploid hybrids. The tetraploid has largely 4-lobed pollen, while the diploid has 3-lobed pollen. Since each complex is represented twice, more pairs might be expected. Renner states that there is no regular pairing of the identical chromosomes such as would be found in an amphidiploid.

Lutz (1912) and Renner (1933) have shown also that two phenotypically different hemi-gigas forms occur in the progeny of lamarckiana. These are triploids. One type has one velans and two gaudens complexes, the other two velans and one gaudens. They originated usually from a diploid egg, but occasionally from a diploid sperm.

A few haploids have been found, but only in the progeny of species with 7 pairs (Emerson 1929, Catcheside 1932). An occasional rod-shaped bivalent was found in the haploid. This evidence for the presence of duplication was used as the basis for one of several proposals by Catcheside as to the origin of new interchanges.

Steps in defining the seven chromosomes and their ends

As mentioned earlier, certain races of Oenothera have 7 chromosome pairs at meiosis. One of these races, O. hookeri, was selected as a standard by Emerson and Sturtevant (1931), with no implication that it might be the ancestral type. Its complex was designated "^{h}hookeri", that is, "haplo-hookeri". The "h" superscript was used by Renner to designate the complex present in double dose in a race homozygous for a complex. The ends of the ^{h}hookeri set of chromosomes were numbered arbitrarily as follows:

1.2 3.4 5.6 7.8 9.10 11.12 13.14.

The following crosses and analyses illustrate the methods used by Emerson and Sturtevant (1931) to define the different chromosomes of ^{h}hookeri and the end-segments. The first step was to select combinations of complexes that gave a ring of four with ^{h}hookeri, and therefore, differed by one interchange. One of these was flavens. ^{h}hookeri. The first two chromosomes of ^{h}hookeri, 1.2 and 3.4 were assumed to have exchanged ends .2 and .4, forming the new chromosomes 1.4 and 2.3. Therefore flavens was 1.4 2.3 5.6 7.8 9.10 11.12 13.14.

The velans. ^{h}hookeri combination also formed a ⊙ 4, 5 II.

The next step was to determine the relation between this interchange and the one in flavens by a cytological study of plants carrying the velans. flavens complexes. These had 2 ⊙ 4 + 3 II, indicating that the two chromosomes interchanged in velans were different

from the two interchanged in flavens. The 6. and .8 ends of chromosomes 5.6 and 7.8 were arbitrarily assumed to have interchanged to form the new chromosomes 5.8 and 7.6. Hence, the velans complex would be 1.2 3.4 5.8 7.6 9.10 11.12 13.14.

The next step was to distinguish chromosome 1.2 from 3.4 and 5.6 and 7.8. Combinations with gaudens served this purpose. The gaudens. ^{h}hookeri combination formed a ⊙ 10, 2 II; hence gaudens has only two chromosomes that are the same as ^{h}hookeri. Gaudens. velans and gaudens. flavens both formed a ⊙ 12, 1 II. Hence gaudens has a hookeri chromosome not present in velans (either 5.6 or 7.8) and also has one not present in flavens (1.2 or 3.4). Or, to restate it, one of the two hookeri chromosomes which were interchanged in velans was also interchanged in gaudens. Chromosomes 1.2 and 5.6 were selected as the two chromosomes in ^{h}hookeri which were not involved in gaudens.

Definitions: On the basis of the above information, chromosome 1.2 was defined as the one present in ^{h}hookeri and gaudens but not in flavens, and 3.4 was defined as the one present in ^{h}hookeri, but not in gaudens or flavens.

Other combinations were used to define the ends of chromosomes 1.2 and 3.4; as follows:

albicans. ^{h}hookeri formed no pairs, albicans. flavens formed a ⊙ 12, 1 II. The pair indicates that one of the interchanged chromosomes in flavens is the same as one in albicans, and therefore, is either 1.4 or 2.3. Selecting 1.4 as the one, the ends were defined as follows:

.1 in the pair of flavens. albicans

.2 in the ring of flavens. albicans

.3 in the ring of flavens. albicans

.4 in the pair of flavens. albicans.

Other combinations were used by them to define the other chromosomes and their ends.

The above examples illustrate the method by which homologies may be established and how other complexes might be analyzed. Extensive analyses were reported by Cleland (1937, 1940) and others. The crosses to produce the combinations of complexes needed for the cytological analysis must be planned with due regard to the information about transmission of the complexes. For example, flavens. ^{h}hookeri might be obtained from the cross of O. hookeri x O. suaveolens ♂, velans. ^{h}hookeri from the cross of O. lamarckiana (one strain) x O. hookeri; but not from the reciprocal crosses.

Examples using the observed configurations in certain hybrids to predict the configurations expected in other combinations of the complexes may be found also in Emerson and Sturtevant (1931), Cleland (1932, 1933, 1936) and others. In certain cases conclusions about the chromosome formulas of a certain race were arrived at by using the cytological information from several hybrids, each of which had a large ring. By complicated reasoning and by listing the remaining possibilities as to end arrangements after the result of each additional intercross was known, they were able to predict the results from other crosses. Cytological observations in most cases agreed with the predictions. In the few exceptions, the cause was found in erroneous original observations on which the prediction had been made. In barley, Chang (1959) found that the same set of interchange testers gave different results in crosses with two interchange lines in which chromosome 1 had been added to the ring by irradiating a line with interchanges involving 3, 5 and 7. How this difficulty can be avoided in Oenothera is not known to me. It is possible that the results still lead to the same conclusions, as they did in barley. Problem 25, p. 359 is an example of this.

The combinations of ends for the seven chromosomes of 398 complexes in Oenothera have been determined by Cleland and his associates (unpublished). The frequencies of the different combinations are shown in Table 45.

Table 45. Frequencies of occurrence of the 91 kinds of chromosomes among the complexes whose segmental arrangements have been completely analyzed by Cleland and associates (unpub.).

End Assoc.	Freq.	End Assoc.	Freq.	End Assoc.	Freq.	End Assoc.	Freq.
1.2	150	3.4	122	5.9	36	8.10	1
1.3	3	3.5	9	5.10	28	8.11	11
1.4	117	3.6	9	5.11	10	8.12	26
1.5	3	3.7	22	5.12	13	8.13	25
1.6	28	3.8	16	5.13	6	8.14	32
1.7	13	3.9	20	5.14	84		
1.8	2	3.10	11			9.10	39
1.9	10	3.11	9	6.7	25	9.11	29
1.10	12	3.12	27	6.8	21	9.12	16
1.11	3	3.13	10	6.9	25	9.13	15
1.12	5	3.14	4	6.10	16	9.14	26
1.13	32			6.11	8		
1.14	20	4.5	3	6.12	43	10.11	54
		4.6	10	6.13	81	10.12	10
2.3	136	4.7	8	6.14	5	10.13	8
2.4	3	4.8	6			10.14	8
2.5	25	4.9	13	7.8	64		
2.6	15	4.10	44	7.9	3	11.12	211
2.7	15	4.11	10	7.10	155	11.13	25
2.8	9	4.12	5	7.11	17	11.14	4
2.9	8	4.13	24	7.12	15		
2.10	12	4.14	33	7.13	3	12.13	14
2.11	7			7.14	16	12.14	5
2.12	8	5.6	112				
2.13	2	5.7	42	8.9	158	13.14	153
2.14	8	5.8	27				
						Total	2786

Note that the 91 different combinations of the 14 ends have been found. Had the combinations of ends occurred at random, the frequency of any one of the 91 should have been 30.6 with a total frequency for any 7 chromosomes of 214.2 or 7.7% of the total. The frequencies for the chromosomes found in a few of the complexes are shown in Table 46.

Table 46. Total frequencies for sets of 7 chromosomes corresponding to several different complexes based on the frequencies in Table 45.

								Total freq.	% of total 2786
$h_{hookeri}$ chromosomes:	1.2	3.4	5.6	7.8	9.10	11.12	13.14		
obs. freq.	150	122	112	64	39	211	153	851	30.5
$h_{johansen}$ chromosomes:	1.2	3.4	5.6	7.10	9.8	11.12	13.14		
obs. freq.	150	122	112	155	158	211	153	1061	38.1
velans chromosomes:	1.2	3.4	5.8	7.6	9.10	11.12	13.14		
obs. freq.	150	122	27	25	39	211	153	727	26.1
gaudens chromosomes:	1.2	4.12	5.6	7.14	8.14	13.10	3.9		
obs. freq.	150	5	112	17	32	8	20	344	12.3

The one with the highest total frequency is ^{h}johansen, 1061 or 38.1% of the total, far in excess of that expected for randomness. Hence, the ^{h}johansen set was the original 7-chromosome ancestor in the evolution of the Oenothera races. The exchange between 1.2 and 3.4 to form 1.4 and 3.2 occurred very early in the evolution of certain groups. Of the other combinations of ends, these two (1.2 and 3.2) have the highest frequencies.

From their survey of the frequencies, they concluded that any end can become associated with any other end, and that there is no evidence that one segmental association can arise more easily than others.

Chiasma frequencies

Harte (1953, 1954, 1954a) found that the frequency of terminal chiasmata was less within the ⊙12 than in the bivalent, based on two hybrids. The same was found in hybrids with a ⊙4, but different complex-combinations with the same chromosome formula gave different frequencies. Also, structural homozygotes and bivalent chromosomes have a higher frequency of non-terminal chiasmata than do the multipartite configurations (Hoffmann 1954). Also, these chiasmata are more frequent in some complex-combinations than in others.

Linkage studies in *Oenothera*

The linkage studies reported by Renner (1925, 1928), Emerson (1930, 1931, b), Shull (1923, 1927, 1928), Sturtevant (1931a) and others have been reviewed by Emerson and Sturtevant (1932). Additional data were reported by Oehlkers (1933) and Renner (1958a).

A list of the genes which have been used in linkage tests is given in Table 47. The associations indicated are based on those races in which that chromosome was present as a separate pair, and the marker inherited independently of those in the ring. This is the result expected if each of the genes is closely linked to an interchange point in the ring. Ordinarily the number of linkage groups corresponds to the number of separate rings and pairs which are genetically marked. The two exceptions are a factor for small flower size (Co) and the factor for brevistylis (br) which are linked with each other with about 15% recombination, but have shown no linkage with any of the other known factors even in plants with a ring of 14 chromosomes. There have been two cases where the F_1 showed one more linkage group than there were separate chromosome groups, but both involved either Co or br. Apparently Co and br are too far from the interchange point to show linkage. Catcheside (1954) has located Co in the .12 chromosome end by using trisomics derived from O. blandina.

With the exception noted above, most of the linked genes show very little crossing over even when they are in paired chromosomes. In the 1.2 chromosome, Shull (1927) found very little crossing over between old gold (v), bullata (bu) and double flowers (sp). Emerson and Sturtevant (1932) reported very little between R and v. In the 3.4 chromosome, Emerson (1932a) found linkage between s and n, 8% between P and s. When this chromosome was in a ring of 10, there was about 5% recombination between p and s. Whenever plants carrying the genetic crossover were checked cytologically, they had the same configurations as their non-crossover sibs. Hence, the crossovers had not occurred in a differential segment. Also, the genes must have been located in the end or interchanged segments.

Emerson (1931b) also reported the results of a 3-point linkage test in plants with sulfurens. franciscana (⊙10) in which each marker was probably located on a different