FURTHER DATA BEARING UPON CIRCLE-FORMATION IN OENOTHERA, ITS CAUSE AND ITS GENETICAL EFFECT¹

RALPH E. CLELAND

Goucher College, Baltimore, Maryland

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INTRODUCTION

In the following pages, data will be presented which bear upon two of the major problems of *Oenothera* research, (1) the genetical effect of

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chromosome cohesion in *Oenothera*, and (2) the cause of chromosome cohesion.

(1) The "chromosome cohesion theory" (see CLELAND 1923, 1924, 1926, 1928, OEHLKERS 1926, CLELAND and OEHLKERS 1929, 1930, EMERSON 1929, 1930, 1931) is to the effect that the union of chromosomes end to end to form circles in diakinesis, and the distribution of adjacent chromosomes to opposite poles in anaphase is a mechanism which is bound to bring about the genetical linkage of genes residing in different chromosomes, so long as these chromosomes are a part of a single circle. This, therefore, is the mechanism responsible for the formation of the so-called "Renner complex." If this theory be correct, then each circle is the basis of a large or super-linkage group, and the number of linkage groups in any form will be equal to the number of chromosome groups. For example, a form with a circle of 10 chromosomes and 2 pairs has one super-linkage group and 2 small, normal linkage groups.

A method of testing this theory exists, for, if it be correct, then one should find a correlation between breeding behavior and chromosome configuration: forms with all of their chromosomes in a circle should breed true, and those with but few pairs should show only minor segregations; while those with a relatively large number of chromosome groups should present a correspondingly greater amount of splitting in their progenies. Evidence that such a correlation does exist has been furnished by OEHLKERS (1926), CLELAND and OEHLKERS (1929, 1930) and EMERSON (1929, 1930, 1931). Further data in support of this correlation will be presented in this paper.

(2) There is a strong indication that segmental interchange is responsible for circle formation in *Oenothera*, as in other organisms. BELLING formulated this theory in 1927. It is to the effect that homologous ends of chromosomes attract one another in diakinesis; that non-homologous chromosomes may exchange end segments occasionally, and that when this occurs the attraction between homologous segments will bring about the union of non-homologous chromosomes to form a chain or circle. The theory has been applied specifically to *Oenothera* by HÅKANSSON (1928, 1930), DARLINGTON (1929), EMERSON (1931) and CLELAND and BLAKESLEE (1930, 1931). The last mentioned have been able to make successful predictions with respect to the chromosome configurations of certain hybrids on the basis of this theory which could not have been made upon any other basis, thus subjecting the theory to a critical test. For the method by which these predictions have been made, the reader is referred to the papers cited.

In the present paper, the results of certain additional predictions will be given, all of which are confirmatory of the theory of segmental interchange.

SOURCES OF MATERIAL

Oe. muricata. Material was derived from seed sent by Professor DE VRIES in 1925 (Lunteren 1923 $\times$ s.). I have grown cultures in selfed line in 1926, 1929 and 1930.

Hookeri de Vries. This strain is from seed received from DE VRIES in 1925 (Lunteren 1923 $\times$ s.). It represents the original line collected in the vicinity of Berkeley, California in 1904 by DE VRIES. I have grown generations in selfed line in 1926, 1929 and 1930.

r-Lamarckiana. Seed was received in 1925 from Professor RENNER (Renner 1925.162 $\times$ s.). The strain came originally from HERIBERT-NILSSON. I have grown cultures in selfed line in 1926, 1929 and 1930.

suaveolens. This line comes from seed sent me by RENNER in 1926 (Renner 1925.86 $\times$ s.). RENNER received the strain originally from DE VRIES. I have grown generations in selfed line in 1926, 1929 and 1930.

grandiflora. This is the strain grown by Professor SHULL as "*grandiflora* B," received originally from DAVIS. The crosses between this *grandiflora* and *franciscana* were made by SHULL in 1924, and they, together with the parent species, were examined cytologically by the writer in 1925.

franciscana. Two strains have been used. One of these, *franciscana* "Shull," is derived from rosettes sent by Professor SHULL in 1925 (Shull 2469 $\times$ s.). This is a selfed line from seed supplied by DAVIS, apparently from his strain *franciscana* B. I have grown cultures from selfed plants in 1925, 1929 and 1930, the race being entirely uniform.

The second strain was received from DE VRIES in 1925 (Lunteren 1923 $\times$ s.). This is a selfed line begun in 1915 from seed sent to DE VRIES by BARTLETT. It therefore had the same origin as DAVIS' *franciscana* B. I have grown cultures in selfed line in 1926, 1929 and 1930, the race being entirely uniform.

FIELD AND CYTOLOGICAL DATA

1930:24 *Oe. (muricata* $\times$ *Hookeri* deV.) (1929:3, pl. 5 $\times$ 1929:14, pl. 9)

Out of 161 seeds sown in Petri dishes, 115 germinated, 85 seedlings were transferred to soil, and 50 plants were set out in the field. These were all uniform, and typical *rigidae*, except plant number 42, which was a metacline. The ungerminated seeds included 19 with embryos.

rigens $\cdot$ ^h*Hookeri*

This complex-combination was first briefly described by DE VRIES (1913), and has been discussed and figured by RENNER (1925).

rigens influence was seen in the color of leaf, stem, bud and fruit; in the near-absence of red on the basal part of the stem; in the thickness of leaves, shape of open flowers, and size of petals in relation to anthers. Plants were intermediate between the parents in habit, amount of red at tips, breadth of the floral tip, stigma-lobe thickness, style length, bud and flower size.

Eleven fixations were made from 3 plants, each fixation from a single plant. The chromosome configuration was found to be a circle of 6 and 4 pairs in all 3 plants. The configuration was seen intact in a total of 66 cells. No exceptional configurations were found, and very little disintegration of the chains was observed.

curvans · ^h*Hookeri*

The presence of metacline hybrids in this cross has been described by RENNER (1925). A single plant of this composition appeared in the culture. It was a combined sectorial and periclinal chimaera. Two-thirds of the plant was in the nature of a periclinal chimaera with bright yellow-white leaf edges, or in some cases with one side of the leaf devoid of chlorophyll (compare RENNER 1924, 1925). The other one-third was wholly green. This plant differed, furthermore, from the other individuals in the family in having narrower, darker green, more irregularly sinuous and more wavy leaves; shinier stems, bent at the tip, with bright red pigmentation below, and more red at the tip, as well as more red papillae; bracts thinner, flowering tip less flattened; buds not so stout, with red papillae on ovary and cone, red pigmentation on hypanthium as well as cone, long spreading hairs and sepal tips widely separated; fruits more slender, greener, and less hairy.

From the 6 fixations taken from this plant, a sufficient amount of material was obtained to demonstrate the presence of a circle of 6, circle of 8 as the chromosome configuration. A total of 28 cells in which the configuration was intact was noted. In addition, a number of cells was found in which the chains had become more or less broken up. In all such cases, however, the configuration could be interpreted as a circle of 6, circle of 8, and no cases were observed in which any other configuration was certainly present.

1930:23 *Oe. (Hookeri deV. × muricata)* (1929:14, pl. 9 × 1929:3, pl. 5)

Out of 213 seeds sown in Petri dishes, only 66 germinated, of which 48 were transferred to soil and 27 came up, all with pale green cotyledons. These all died early because of the lack of chlorophyll.

The failure of *Hookeri* plastids to function in the presence of the ^a*Hookeri. curvans* gene combination has been noted previously by RENNER (1924, 1925).

1930:29 *Oe. (suaveolens* × *muricata*) (1929:11, pl. 6 × 1929:3, pl. 10)

RENNER has shown that only the *albata* is viable in this cross (1918, 1925). *Oe. suaveolens* plastids fail to function in the presence of the *flavens* · *curvans* gene combination.

Only 42 seeds were sown in Petri dishes, of which 14 germinated. Of the ungerminated seeds, 14 were empty and 14 had embryos. Of those which germinated, only 6 came up when transferred to soil, 5 of which were extremely chlorotic and soon perished. One green seedling only was left, which grew to be a large rosette but sent up no flowering shoot. It was no doubt an *albata*.

1930:30 *Oe. (muricata* × *suaveolens*) (1929:3, pl. 10 × 1929:11, pl. 6)

From 183 seeds sown in Petri dishes, 152 germinated and 146 were transferred to seed pans and came up. Of the 31 ungerminated seeds, 14 had embryos and 27 were empty. The plants were quite uniform except for one individual. This plant and 49 normals were set out in the field. Except for the one aberrant plant, the culture remained uniform throughout the season, and corresponded to the description for *rigens* · *flavens* given by RENNER (1918, p. 644).

The aberrant plant was a dwarf, scarcely 30 cm high, with an eccentric stalk, and basal branches confined to one side of the plant. The leaves were proportionately broader and less pointed than in the normal, the bracts relatively larger, in comparison with the very small fruits. The stem lacked red papillae and red pigmentation at the tip. The buds were wholly green, with sparse bristly hairs, very stout, with sepal tips widely separated. The plant evidently contained, at least in part, the *curvans* complex, but was clearly not a typical *curvans* · *flavens*. It turned out to have 15 chromosomes.

Material was collected from 4 plants of the normal *rigens* · *flavens* type. These were in agreement in showing a circle of 4, circle of 6 and 2 pairs as their configuration. Altogether, 68 cells showing the complete configuration were observed, a number which represents but a small proportion of those which might have been studied on the slides examined had the search been prolonged. In better fixed regions there was found very little disintegration of the chains, and no evidence of abnormal configurations.

Oe. franciscana deV. and *Oe. franciscana* Sh.

In 1922, the writer published an account of the cytology of *Oe. franciscana* Bartlett, demonstrating the presence of a circle of 4 chromosomes and 5 pairs in the pollen mother cells of 4 plants. In 1925, however, the *franciscana* of SHULL was found to have nothing but paired chromosomes (CLELAND 1928).

In view of this discrepancy, cultures of both *franciscana* Shull and *franciscana* de Vries have been grown in 1929 and 1930, and have been investigated cytologically. Fixations during 1929 were made from 3 plants of *franciscana* deV. and 5 plants of *franciscana* Sh. During 1930, material was examined from 5 plants of *franciscana* deV. and 3 plants of *franciscana* Sh. All of these plants without exception showed 7 pairs of chromosomes in their pollen mother cells. No record was kept of the number of cells observed in the 1929 material, although the number was quite large. In 1930, 56 cells of *franciscana* Sh., representing all 3 plants, and 80 cells of *franciscana* deV., representing all 5 plants, were clearly observed to have 7 pairs. These numbers could have been greatly increased, had it been thought necessary.

Especial attention was paid to late second contraction and early diakinesis stages in these studies, in view of the published statement by KULKARNI (1929), who found that a circle of 4 was present in early diakinesis, which soon broke up, so that mid and late diakinesis presented 7 pairs. In none of my material, however, was I able to find a circle of 4, even in early stages. I am forced to the conclusion that such a circle is not present, and that the normal configuration of these strains is 7 pairs.

The difference in chromosome configuration between the form studied in 1922 and these later forms may perhaps be correlated with slight morphological differences. Thus, according to DAVIS' description of *franciscana* B (1916), the cone of the mature unopened bud is streaked with bands of red, whereas in my material the scarlet or salmon-red pigmentation is uniformly distributed over the body of the sepals, except for the calyx tips and for faint greenish lines separating the sepals. Furthermore, my material shows a considerable amount of diffuse anthocyan pigment in the stems, especially below, but also at the extreme tips. DAVIS' description says nothing of the red pigmentation at the tips of the stems, referring to the stems as green. Also, DAVIS' plants showed strong central shoots, with shorter side branches, whereas mine have consistently shown only side branches, central shoots having appeared but rarely. While I am by no means certain that these differences are significant, and that

they may not be correlated with differences in environment, it is possible that my material with its 7 pairs is slightly different genetically from the material which has a circle of 4. (In a recent letter DAVIS informs me that his present strain of *franciscana* B agrees with mine in bud color but has a very strong central shoot, and the tips of the stems are green.)

While the reason for this cytological divergence has not been ascertained, it seems to me that there is a possible explanation. *Oe. franciscana* is an alethal species, and consequently would not be expected to breed true for a circle of chromosomes if it had one. Assuming that the circle has been produced by segmental interchange, and that the chromosomes have definite positions within the circle, due to the attraction between like ends of non-homologous chromosomes, then, with adjacent chromosomes passing to opposite poles, two classes of gametes would be formed with respect to the chromosomes comprising the circle. Any recombination being possible, the progeny of a selfed plant would therefore contain two classes of plants with 7 pairs, totalling one-half of the progeny, and one class with a circle of 4, comprising the other half. In short, all plants heterozygous for the chromosome complexes produced from the circle should have the circle; those homozygous for one or other of the complexes should have 7 pairs.

The small size of the circle in *franciscana*, and the absence of lethals, suggests that the circle is of comparatively recent origin, and that the chromosomes comprising the opposing complexes formed by the circle are relatively similar genetically. Consequently, it may well be that the segregation in respect to complexes in this case is accompanied by a phenotypic segregation of so minor a nature as to escape attention. Apparently, then, in the case of both of my strains of *franciscana*, some worker has unwittingly chosen for the purpose of selfing one of the homozygous segregates with 7 pairs, instead of a circle-bearing heterozygote, and so has established a race without circles. This supposition is strengthened by the fact that, whereas both of my strains give 7 pairs with *Hookeri* in reciprocal crosses, the strain of STURTEVANT and EMERSON, which is also derived from DAVIS' *franciscana* B, and which has 7 pairs, gives a circle of 4 with *Hookeri*, and would consequently give a circle of 4 with my strains of *franciscana*. There is at least a strong probability that I have one of the two possible homozygous segregates from the circle-bearing *franciscana*, and STURTEVANT and EMERSON have the other. It will be an easy matter to bring these two together, and compare the resultant hybrid phenotypically with the original description of *franciscana*.

1925:6 *Oe. (grandiflora B* $\times$ *franciscana Sh.)* (=Shull 2473)

This culture was grown at the UNIVERSITY OF PENNSYLVANIA in 1925, from rosettes kindly sent me by Professor SHULL. The plants were uniform. They resembled *grandiflora* B. in the presence of a strong central shoot, evanescent rosette, absence of rosette branches, in color and hairiness of leaves, relative absence of pigment on stems, and green buds. They were like *franciscana* in the presence of red papillae, in the hairiness of buds and fruits, and showed the influence of *franciscana* in size and shape of the leaves. It should be noted, furthermore, that the plants were grown in a relatively shady situation, and it is possible that had they been placed in a more open position, they might have shown to some extent the production of red pigment in the stem tips and bud cones.

Collections were made from 5 plants. A total of 13 fixations was obtained, each fixation from a single plant. The chromosome configuration of all 5 plants was determined, and was found to be a circle of 4 and 5 pairs (see CLELAND 1928). A total of 61 cells showing the configuration was recorded. The fact that all the plants were alike in configuration, coupled with the phenotypic uniformity of the culture, strengthens the conclusion that both parents produce but one type of gamete, and that but one kind of hybrid is therefore possible between them.

Oe. (franciscana Sh. $\times$ *grandiflora B)* (=Shull 2474)

This hybrid was grown simultaneously with its reciprocal by Professor SHULL, at Princeton, New Jersey. The plants were for the most part chlorotic, so that the great majority died either in the pots or in the field. Only 13 plants out of 129 came into bloom. So far as could be determined, however, there were no differences between this culture and its reciprocal which could not be ascribed to the greater vigor of the reciprocal resulting from its freedom from chlorosis.

Material was collected from the greenest individuals of this culture. Owing to the relative scarcity of buds, however, two or more plants were included in each fixation. As all fixations were made on the same day, each collection represents a different set of plants. Material was examined from 3 collections, representing at least 3 plants. All buds were found to have the configuration characteristic of the reciprocal, namely a circle of 4 and 5 pairs. In all, 37 complete nuclei were observed which showed the normal configuration.

1930:22 *Oe. (franciscana deV. × Hookeri deV.)*
(1929:5, pl. 1 × 1929:14, pl. 2)

Of the 150 seeds sown in Petri dishes, 149 germinated, 30 seedlings were transferred to soil and 27 plants were set out and grew to maturity. The seed which failed to germinate was devoid of an embryo.

Plants resembled *franciscana* in leaf breadth and shape, bud shape, color and hairiness; they resembled *Hookeri* in the flatness of the leaves, midrib color, marginal leaf color, stem color and compactness of floral tip. Other characters were either common to the parents, or intermediate in the hybrid.

A total of 14 fixations was obtained from 5 plants of this culture, each fixation from a single plant. The proper stages were obtained from 4 plants, which showed entire uniformity of cytological behavior, with 7 pairs of chromosomes present during diakinesis. A total of 47 pollen mother cells showed beyond question the normal arrangement.

1930:21 *Oe. (Hookeri deV. × franciscana deV.)*
(1929:14, pl. 9 × 1929:5, pl. 1)

All of the 133 seeds sown in Petri dishes germinated. Of these, 30 were transferred to soil, and 15 plants were brought to maturity. These plants were uniform, and resembled their reciprocal in every way.

Fixations were made from 9 plants, a total of 15 collections, each from a single plant. Material from 8 of these was found to have the proper stages. Seven plants were in agreement as to chromosome configuration, having 7 pairs like the reciprocal. I observed 92 cells in which all 7 pairs could be clearly seen. One plant, however, showed a circle of 4 in all pollen mother cells. Only one fixation was made from this plant, and only one bud was obtained in the proper stage, so that it is not certain whether this configuration was characteristic of the whole plant or only of a single bud or branch. The probabilities are that this is the configuration of the whole plant, and that the circle arose through a segmental interchange which took place at the formation of one of the germ cells which produced this plant. The preponderance of plants with 7 pairs, however, and the fact that the reciprocal showed this configuration also, indicate that perfect pairing is the normal condition in this combination, and that one plant was exceptional in this respect.

The fact that the reciprocals in crosses with the homozygous *Hookeri* deV. are uniform and identical with each other in all respects, phenotypi-

cally and cytologically, indicates that *franciscana* deV. is also practically or entirely homozygous.

1930:20 Oe. (*franciscana* Sh. $\times$ *Hookeri* deV.)
(1929:4, pl. 9 $\times$ 1929:14, pl. 9)

Of the 162 seeds sown in Petri dishes, 122 germinated and 19 plants were brought to maturity. The 40 seeds which failed to germinate were all devoid of embryos. The culture was uniform, and was practically indistinguishable from *franciscana* deV. $\times$ *Hookeri* deV.

Collections were made separately from 3 plants, 3 collections from each plant. All three plants showed the uniform presence of 7 pairs of chromosomes in their pollen mother cells, a total of 57 cells being noted with this arrangement. No other configuration was observed. Cytologically, as well as phenotypically, therefore, this culture resembled the corresponding cross, *franciscana* deV. $\times$ *Hookeri* deV.

1930:19 Oe. (*Hookeri* deV. $\times$ *franciscana* Sh.)
(1929:14, pl. 9 $\times$ 1929:4, pl. 2)

From 210 seeds sown in Petri dishes, 197 germinated, 30 seedlings were transferred to soil, and 18 plants were brought to maturity. The 13 seeds which failed to germinate were found to have no embryos. This culture fully resembled its reciprocal in appearance, and hence was indistinguishable from the corresponding culture involving *franciscana* deV.

Eleven fixations were made from a total of 5 plants, each fixation being confined to a single plant. Material of only 3 plants was sectioned and studied. The three showed 7 pairs of chromosomes in every one of the 51 complete pollen mother cells examined.

1930:40 Oe. (*franciscana* deV. $\times$ *muricata*)
(1929:5, pl. 2 $\times$ 1929:3, pl. 10)

HOEPPENER and RENNER (1929) report that *franciscana* $\times$ *muricata* results in nothing but pale seedlings which fail to grow. I was not able even to secure germination, although the seeds had embryos for the most part. Had pressure been used, it is possible that they might have germinated.

1930:39 Oe. (*muricata* $\times$ *franciscana* deV.)
(1929:3, pl. 10 $\times$ 1929:5, pl. 2)

Of the 109 seeds sown in Petri dishes, 56 germinated and 34 plants were set out in the field. Of the 53 seeds which did not germinate, 50 were devoid of visible embryos. One plant which reached maturity was un-

doubtedly a typical *franciscana*, included by mistake. It had 7 pairs of chromosomes. Another plant was probably a metacline. It was small, with narrow toothless leaves, and did not develop beyond the rosette condition. Except for these two plants, the culture was uniform and similar to *muricata* × *Hookeri* deV. It differed from the latter chiefly in having blunter, broader, decidedly more wavy leaves, with margins less evenly toothed, midribs white, and in having slightly larger flowers and slightly less color on the bud-cones.

These plants were clear *rigidae* in color of foliage, stem and bud, and in shape of bud and flower. The influence of *franciscana* was most clearly seen in the waviness of the leaves, the more delicate stems, red pigment at the stem tips, larger flowers, narrower stigma lobes, and slight trace of red pigment on the lower half of the bud-cone.

Excluding the *franciscana*-like plant which had 7 pairs of chromosomes, fixations were obtained from 4 plants, a total of 11 fixations having been made. All 4 plants were uniform as to chromosome configuration, with a circle of 6 and 4 pairs in every pollen mother cell. The total number of complete pollen mother cells seen was 69.

1930:38 Oe. (*franciscana* Sh. × *muricata*)
(1929:4, pl. 2 × 1929:3, pl. 10)

From 108 seeds sown in Petri dishes, 68 germinated and 30 seedlings were transferred to earth, of which 26 came up. Of those which did not germinate, 37 had embryos. All of the seedlings which came up were yellowish, with scarcely a trace of chlorophyll, and all died a few days after their appearance. They were all of the composition *^hfranciscana* · *curvans*.

1930:37 Oe. (*muricata* × *franciscana* Sh.)
(1929:3, pl. 10 × 1929:4, pl. 2)

Of 121 seeds sown in Petri dishes, 68 germinated and 53 failed to germinate. Only one of the latter had an embryo. Of the 31 plants set out in the field, 4 plants were metaclines, *curvans* · *^hfranciscana*, and the rest were *rigidae*, *rigens* · *^hfranciscana*.

rigens · *^hfranciscana* Sh.

The plants of this composition were uniform and resembled the corresponding *rigens* · *^hfranciscana* deV. very closely. The only differences that were noted were perhaps of doubtful significance, namely, one plant of *rigens* · *^hfranciscana* Sh. had a central shoot, which none of the corresponding culture had, and the culture showed as a whole rosette branches with

noticeably fewer secondary branches than were found in *rigens* · ^h*franciscana* deV.

From three plants of this composition, 11 fixations were obtained, each from a single plant. All 3 plants showed in every complete pollen mother cell a circle of 6 and 4 pairs (59 complete cells were noted), the configuration found in the corresponding *franciscana* deV. cross.

curvans · ^h*franciscana* Sh.

The 4 plants of this composition were alike except for the degree and nature of the variegation which they displayed and the consequent differences in general vigor. Features possessed in common were as follows:

Leaves dark green, narrower than in *rigida*, wavy, very irregularly sinuous-toothed, with white midribs and red-toothed margins. *Stems* all lateral, quite red below, greenish above, tips decidedly reddened, red papillae numerous; long hairs abundant, fine felt also present; stems at first distinctly bent over at tips, this character becoming obscured toward the end of the flowering period. *Bracts* reddened beneath, spreading, curly-wavy, slightly exceeding buds when young, creating a flat-topped floral tip. *Buds* with numerous erect bristly hairs, but lacking the fine felt of the *rigida*; red papillae on ovary, diffuse pigmentation on hypanthium and cone; cone color about as in *Hookeri* deV. *Sepal* tips separated, sometimes widely. *Petals* about as broad as long, deep yellow, with entire margin, and hardly any prominence in center of notch. *Stigma* slightly touched by anthers. *Flower* 2.5–3.5 cm across. *Fruit* longer, narrower than *franciscana* Sh. or *rigida*, tapering, with some diffuse red.

Each of the 4 plants represented a different condition in respect to the matter of variegation.

Plant number 4 wholly green, but the last of the 4 to come into flower.

Plant number 7, a peculiar type of chimaera. About one-half of the plant perfectly green, the other half a periclinal chimaera, with green outside and yellow inside. Fruits on these branches for the most part yellowish, except that edges of carpels were occasionally dark green, thus creating thin green stripes along the fruits. *Sepals*, like foliage leaves, yellow-green with dark green borders. *Stigma* lobes a normal green when young. The division line between green and variegated portion of the plant went through the middle of a lateral branch on either side of the rosette.

Plant number 16 like number 7, except that the nature of the chimaera was reversed. One-half the plant wholly green, the other half a periclinal chimaera, but the yellow outside, and green inside. Fruits wholly green, yellow stripes hardly noticeable at edges of the carpels. *Sepals* green with

yellow borders, stigma lobes yellow when young, instead of green as normally. The division line between green and variegated portions of the plant passed between lateral branches.

Plant number 20 variegated throughout, distribution of yellow and green portions highly irregular. Many leaves wholly yellow on one side, green on other. Others had no green at all, while a few were mostly green. Owing to the small amount of green tissue, this plant remained a small rosette.

It is evident that *franciscana* plastids are no more able to function in the presence of the ^h*franciscana*·*curvans* gene combination than are *Hookeri* plastids, when in the presence of the ^h*Hookeri*·*curvans* combination. Plants containing either of these gene combinations, and receiving *franciscana* or *Hookeri* plastids, respectively, through the pollen, will be more or less variegated (see RENNER 1924).

Fixations were made from plants number 4, 7 and 16. Each fixation was from a single plant, and some of the fixations were confined to green portions, others to variegated portions of the plants. All three plants were found to have a circle of 6, circle of 8 in their pollen mother cells, the same chromosome configuration being found in green and variegated portions. No certain deviations from the normal configuration were found, although there was some tendency on the part of the circles to shatter. A detailed study was restricted to plants 7 and 16, and yielded a total of 40 cells showing beyond question the normal configuration.

1930:28 Oe. (*franciscana* deV. × *suaveolens*)
(1929:5, pl. 2 × 1929:11, pl. 9)

Of the 57 seeds sown in Petri dishes, only 10 germinated, the rest lacking embryos of sufficient size to be recognized upon pinching the seeds. Those which germinated were transferred to soil, but only 6 came up. Of these, one died in the cotyledon stage, and the remaining five lived until mid-summer, being set out in the field. All were pale green with narrow, slightly crinkled leaves having white midribs. They possessed an insufficient amount of chlorophyll for proper nourishment, and finally succumbed.

The reciprocal of this cross was not grown.

1930:26 Oe. (*franciscana* Sh. × *suaveolens*)
(1929:4, pl. 2 × 1929:11, pl. 9)

Of 144 seeds sown in Petri dishes, 80 germinated, most of the remainder being devoid of recognizable embryos. Of the 30 seeds transferred to soil, 27 came up. These were of a decidedly pale green cast, and lived only long

enough to develop 2 or 3 foliage leaves. They were therefore somewhat more chlorotic and somewhat shorter lived than the corresponding *franciscana* deV. $\times$ *suaveolens* hybrids. Both *franciscana* deV. and *franciscana* Sh. differ from *Hookeri* deV. in their reaction with *flavens*. *Hookeri* gives green plants in the cross *Hookeri* $\times$ *suaveolens*.

1930:25 Oe. (*suaveolens* $\times$ *franciscana* Sh.)
(1929:11, pl. 8 $\times$ 1929:4, pl. 2)

Out of 175 seeds sown in Petri dishes, 142 germinated, and 33 failed to germinate. The latter included only 4 with recognizable embryos. Of the germinated seeds, 45 were transferred to soil, 38 seedlings came up, and 35 plants were set out in the field. Within a month of sowing the distinctions between *flavae* and *albatae* were clear. The former were larger than the latter, with leaves more crinkled and more acute, containing red spots and marginal teeth. There were 25 *flavae* and 10 *albatae* among those set out.

flavens · ^h*franciscana* Sh.

Description. Strong central shoot, as well as well-developed rosette branches, plants 70–80 cm high when mature. *Rosette leaves* rather flat, evenly but slightly crinkled, dark green, broader than those of *albatae*, midribs white, margins and marginal teeth green, marginal hairs appressed, susceptible to mildew. *Bracts* erect when young, considerably longer than buds; later becoming horizontal, reaching to lower part of sepals in anthesis, slightly longer than fruits when mature; slightly reddened beneath. *Stems* dark red below, becoming green above, with green tips; many red papillae, many long hairs, slight down. *Buds* when young softly silky, like *franciscana*, except hairs shorter; anthers show tendency to bend in at tips: when mature, slender, about 7 cm long, hypanthium long (3 cm), green; ovary softly hairy, with few red papillae; cone with red stripes part way up from base, rather sparsely hairy, including some glandular hairs, tips long, delicate, slender, appressed; petals occasionally imperfect, with deep clefts in side; when perfect, with slight prominence in notch. Flowers averaged 4.1 cm across. Style various in length, stigma generally touched by anthers. *Fruit* fairly stout.

Plant number 9 was typical in every way except that it was a sectorial chimaera, half the plant being entirely green and the other half being in the nature of a periclinal chimaera with white leaf margins and green centers. Apparently, a part of the embryo had received a preponderance of *franciscana* plastids, derived through the pollen tube, which were in-

capable of functioning in the presence of the *flavens*·^h*franciscana* gene combination.

Collections were made from 4 plants, 3 collections from each plant. Every pollen mother cell in which all the chromosomes were visible showed a circle of 4 and 5 pairs. No exceptions were observed in 55 complete cells.

albicans·^h*franciscana*

These plants were later in maturing than the *flavae*; in fact only 4 of the 10 sent up flowering shoots, the rest remaining as vigorous rosettes.

Description. No central shoot. *Rosette leaves* proportionally narrower, more crinkled and wavy than in *flava*, deep grayish-green, with white mid-ribs. *Bracts* when young bend out at tips, later become horizontal, shorter than hypanthium in anthesis, little longer than mature fruits; narrower than in *flava* when young, wavier when old. *Stems* barely pinkish below, becoming green above, with abundant red pigment at tips, stouter than in *flava*, more sparsely hairy, with less down; red papillae present, but fewer. *Buds* stout, clear green, or, exceptionally, with slight trace of red on cone, about as hairy as in *flava*; sepal tips thick, erect, more or less appressed; petals lighter yellow than in *flava*, with decided prominence in notch, occasionally showing imperfections in margin. Style rather long, stigma lobes very long. Flowers averaged 4.7 cm across. *Fruit* grayer than in *flava*, about same size and shape.

The more striking differences between *flava* and *albata* are to be found in habit, in stem coloration and thickness, leaf shape and size, bract position when young, which influences the shape of the floral tip (flatter in *albata*, with more exposed buds), bud coloration and thickness, color of fruit, length and stoutness of stigma lobes.

Ten fixations were made, each from a single plant, 4 plants being represented. Material was sufficient to determine the chromosome configuration of 3 plants. All three were in agreement in showing a circle of 14 chromosomes (32 complete nuclei showed the configuration clearly).

1930:34 Oe. (*franciscana* deV. × *r-Lamarckiana*)
(1929:5, pl. 2 × 1929:1, pl. 3)

Out of 329 seeds sown in Petri dishes, all germinated but 18, of which 10 had no visible embryos. The seeds transferred to soil yielded 59 plants, of which 30 were set out in the field. These were all typical *velutinae*, except for two aberrants, which had the appearance of "mutant" types. For some reason, *laetas* were absent. Most of the plants remained in the rosette condition throughout the season.

This culture bears a great resemblance to the corresponding *Hookeri* deV. cross. In habit, the two are much the same, both showing a greater tendency toward the production of the central shoot on the part of late blooming individuals, and a lesser tendency on the part of earlier blooming ones. In both hybrids, the central shoot, when present, has a multitude of small side branches, which bloom about the same time, so that the plant is a mass of closely set blooms when in full flower.

Material was collected from 7 plants, each of the 16 fixations being from a single individual. The chromosome configuration was found to be a circle of 4 and 5 pairs in all 7 individuals. A detailed record was kept for 4 plants, from which a total of 34 complete cells was obtained.

1930:33 *Oe. (r-Lamarckiana* × *franciscana* deV.)
(1929:1, pl. 3 × 1929:5, pl. 3)

From the 149 seeds sown in Petri dishes, 106 germinated, 59 seedlings were transferred to soil and 32 plants set out in the field. Of the seed which failed to germinate 23 had no visible embryo.

A distinction between *laetae* and *velutinae* was early evident, the latter developing a yellowish coloration in varying degree on cotyledons and later leaves, whereas the broader leaves of the former remained dark green, and developed red spots on the foliage.

velans · ^h*franciscana* deV.

Only 11 of the 28 *velutinae* came to maturity. The rest lived throughout the season, but as rosettes. The various rosettes differed in size, and in width and degree of yellowing of the leaves. One plant was wholly green and indistinguishable from the reciprocal hybrid described above. It is clear that the other plants of the culture differed from the reciprocal only in the tendency toward yellowing of the foliage, with consequent reduction in vitality.

Collections were made individually from 9 plants. Microscopic examination was made of 6 plants, and all were found to possess a circle of 4 and 5 pairs. A total of 55 cells showing the complete configuration was noted. This culture agrees therefore with its reciprocal in chromosome configuration.

gaudens · ^h*franciscana* deV.

The *laetae* were 4 in number. One of these remained in the rosette condition, the other 3 bloomed. They resembled the corresponding *gaudens* · ^h*Hookeri* very closely.

Eleven fixations, each from a single plant, were obtained, representing 3

plants. These all had a circle of 10 and 2 pairs (observed in its entirety in 18 cells).

1930:32 *Oe. (franciscana Sh. × r-Lamarckiana)*
(1929:4, pl. 9 × 1929:1, pl. 3)

Of the 126 seeds sown in Petri dishes, 107 germinated, 60 seedlings were transferred to soil and 15 plants were set out in the field. Embryos were lacking in 6 of the seeds which failed to germinate. All the resulting plants were *velutinae*.

These plants were identical in appearance with the corresponding (*franciscana* deV. × *r-Lamarckiana*) *velutina* except that they all finally bloomed. Those which bloomed later, however, developed strong central shoots in contrast to those which bloomed early, in which central shoots were either absent or developed only weakly toward the close of the flowering period of the plant.

A total of 14 fixations was made individually from 6 plants. Material from 5 of these plants was studied cytologically, and all were found to have a circle of 4 and 5 pairs, like the corresponding cross involving *franciscana* deV. Detailed examination was made in the case of 4 plants, a total of 57 complete cells being recorded.

1930:31 *Oe. (r-Lamarckiana × franciscana Sh.)*
(1929:1, pl. 3 × 1929:4, pl. 9)

Out of 172 seeds sown in Petri dishes, 129 germinated, of which 54 were transferred to soil. Many were so weak that they failed to push up through the soil. Those which succeeded were decidedly chlorotic, and some died very shortly. At the time of transplanting to the field only 10 plants remained. These were set out, and all survived the summer, but only one bloomed. All were *velutinae*.

These plants differed from their reciprocal in being chlorotic, and hence smaller and weaker, with narrower leaves, thinner stems and fewer buds. Except for these differences, the reciprocals were no doubt alike. It is worthy of notice that the plastids of *velans* like those of *flavens* are more sensitive to the presence of the *franciscana* Sh. gene complex than they are to that of *franciscana* deV., functioning slightly better with the latter than with the former.

An attempt was made to obtain cytological material from the single plant which bloomed. The proper stages were not obtained, however, and we must be content for the present to infer that the configuration of this hybrid is a circle of 4 and 5 pairs.

DISCUSSION

The genetical effect of chromosome cohesion in Oenothera

According to the chromosome cohesion theory of extensive genetical linkage in *Oenothera*, the genetical effect of the union of chromosomes end to end in diakinesis and the heterotypic metaphase would be to bring about a linkage between genes lying in different chromosomes. If this theory is correct, then the number of linkage groups in any given form should not be greater than the number of chromosome groups which it possesses. So far as the evidence goes at present, indications point toward a close correlation between the number of chromosome groups and the degree of independence existing between genes, forms with larger circles having progenies which show very little or no splitting, those with smaller circles, and hence more chromosome groups, yielding progenies in which more segregation is found. Flower size factors have so far proved the only ones which do not fit in with this interpretation, for in some cases splitting occurs as to flower size, even where the chromosomes are all united into a single chain. Apart from this one exception, however, the trend of evidence is decidedly in favor of the chromosome cohesion theory (OEHLKERS 1926, CLELAND and OEHLKERS 1929, 1930, EMERSON 1929, 1931). Nevertheless, the evidence is not yet as extensive as it should be, and further data are desirable.

In each of the following complex-combinations mentioned above it will be observed that the chromosome configuration is such as to render possible the degree of splitting found in the progeny of this hybrid, on the basis of the theory. The data from the crosses reported in this paper are therefore favorable to the chromosome cohesion theory of extensive genetical linkage.

rigens · ^h*Hookeri* deV.

RENNER has shown that *rigens* and ^h*Hookeri*, when brought into union, are capable of exchanging certain genes. These genes are therefore no longer linked with the complexes to which they normally belong, but have become independent. Such a dissolution of a linkage bond is easily understood when we realize that in *rigens* · ^h*Hookeri* the chromosomes are associated, not in one large circle, but in such a way as to form a circle of 6 chromosomes and 4 pairs. As long as the chromosomes carrying certain *rigens* genes were associated into a single circle (as was the case in *muri-cata*), and were so placed within the circle that they always went to the same pole in the heterotypic anaphase, these genes were linked, genetically speaking. But when these same chromosomes became separated into dis-

tinct groups, then the genes carried by them were capable of independent assortment. In *rigens*·^h*Hookeri* there are 5 independent chromosome groups, and there is therefore the possibility of 5 linkage groups.

Hence, the fact that, in (*muricata* × *Hookeri* deV.) *rigens*·^h*Hookeri*, *R-rigens* and ^h*Hookeri* exchange *R* and *r*, *P S_{tr}* and *P s_{tr}*, *S_{pr}* and *s_{pr}*, and probably certain flower size factors (RENNER 1925, p. 99), is in line with expectations on the basis of the chromosome cohesion theory, but scarcely to be understood on the assumption that their linkage in *muricata* proves them to lie in a single chromosome.

curvans·^h*Hookeri* deV.

RENNER reports (1925, p. 111) that the progeny of *Hookeri-curva* shows splitting in the case of a factor or factors for flower size, as well as the factor pair *S_{pr}*, *s_{pr}*. He found evidence of further splitting also, but this had not been completely analyzed at the time of this publication.

Other forms which have shown a circle of 6, circle of 8 have bred true (with the possible exception of flower size), and have not shown splitting such as has been found to occur in the progeny of ^h*Hookeri*·*curvans*. However, the situation in this hybrid is not comparable to that in the other forms which have so far shown the same configuration, for in this case we are dealing with an unbalanced lethal situation, whereas the other forms had balanced lethals. Since ^h*Hookeri* carries no lethals, it is safe to assume that the ^h*Hookeri* half of each circle in ^h*Hookeri*·*curvans* is lethal free. Just how many lethals there are in *curvans* we do not know. A lethal condition may exist in the *curvans* half of both circles, or only of one circle. If the former be the case, *curvans*·^h*Hookeri* could presumably produce, upon self-pollination, 4 genotypes in the theoretical ratio of 4:2:2:1. If the latter be the actual situation, then we might expect 6 genotypes in the theoretical ratio of 4:2:2:2:1:1. In spite of the fact, therefore, that all of the chromosomes of this form are associated into circles, it is not only possible, but to be expected, that splitting, over and above the segregation of pure *Hookeri*, will occur in a progeny derived by self-pollination.

I may point out in this connection that the *Hookeri-curvae* upon which RENNER's breeding data are based may not have been in every case wholly typical from the standpoint of the gene complexes which they possessed, although they may have had the chromosome configuration typical of *Hookeri-curva*. The published data in regard to splitting in the progeny of *Hookeri-curva* have been derived from the use of 2 *Hookeri-curvae*, (a) one obtained from the cross (*r-Lamarckiana* × *Hookeri*) *r-laeta* F₃ × *muricata*, and (b) the other from (*suaveolens* × *Hookeri*) *flava* × *muricata*.

Oe. (r-Lamarckiana × Hookeri) laeta has a circle of 10 and 2 pairs. If there are any heterozygous genes in the pairs in this hybrid, there is the possibility of independent assortment and consequent exchange of these genes from one complex to the other. This form, having an unbalanced lethal situation, will produce, if selfed, plants with 7 pairs, and plants with a circle of 10, the latter will repeat this behavior if selfed, and so on. (*r-Lamarckiana × Hookeri*) *laeta* F₃ should therefore have a circle of 10, and 2 pairs. With respect to the 4 chromosomes which form the two pairs, however, these may belong all to *gaudens*, or all to ^h*Hookeri*, or some to one, some to the other. Consequently there is at least the theoretical possibility that the ^h*Hookeri* which was united to *curvans* when (*r-Lamarckiana × Hookeri*) *laeta* F₃ was crossed with *muricata* had one or two *gaudens* chromosomes instead of the corresponding ^h*Hookeri* chromosomes. The two chromosomes which have the same arrangement of ends in *gaudens* and ^h*Hookeri*, and are therefore able to pair when these complexes are combined, are 1·2 and 5·6 (see CLELAND and BLAKESLEE 1931). 1·2 contains *R*, *r* and 5·6 contains *S_p*. Since *r-Lamarckiana* was used rather than *R-Lamarckiana* in this pedigree and both ^h*Hookeri* and *rigens* carry *r*, there is no way of knowing whether the ^h*Hookeri* used in this cross was pure ^h*Hookeri* or not (all complexes involved carry *S_p*).

Oe. (suaveolens × Hookeri) flava has a circle of 4 and 5 pairs. Furthermore, RENNER has shown that ^h*Hookeri*·*flavens* and reciprocal give progenies which show a very complex splitting, and which do not include typical ^h*Hookeri* (1925, p. 103). It is quite likely, therefore, that any so-called ^h*Hookeri* produced by this hybrid would have one or more *flavens* chromosomes in place of the corresponding ^h*Hookeri* chromosomes, and would not be an altogether typical ^h*Hookeri*. Consequently, it is altogether probable that the "^h*Hookeri*" which united with a *curvans* complex in (*suaveolens × Hookeri*) *flava* × *muricata* was really a mixture of ^h*Hookeri* and *flavens* genes.

RENNER also mentions having obtained *Hookeri-curvae* in other ways. Thus, they have been obtained as metaclines in the cross *muricata* × *Hookeri* (1925, p. 108). Here, the hybrid obtained should contain pure ^h*Hookeri* and pure *curvans*. They have also been obtained from the cross (*R-muricata* × *Hookeri*) *rigens*·^h*Hookeri* × *R-muricata* (with *r-curvans* pollen) (1925, p. 109). In this case, there is the possibility that the ^h*Hookeri* eggs which functioned contained one or more *rigens* chromosomes, for *rigens*·^h*Hookeri* has a circle of 6 and 4 pairs. It is not surprising therefore to find that the *Hookeri-curvae* obtained from this cross were not uniform but included 18 *S_{tr}*, *R*; 18 *S_{tr}*, *r*; 16 *s_{tr}*, *R*; and 21 *s_{tr}*, *r*. The *R* and *s_{tr}* found in the eggs

came in this case from *rigens*, so that the presence of *R* and the absence of *S_{tr}* in some of the *Hookeri-curvae* show that an interchange had occurred between *rigens* and ^h*Hookeri*, probably due to the transfer of whole chromosomes from one complex to the other.

Once again a *Hookeri-curva* was obtained from the cross *M-flava* (*flavens* · ^h*Hookeri*) × *r-muricata* (1925, p. 111). In this case it is impossible to make any statement with regard to the purity of the *flavens* complex involved inasmuch as we do not know the chromosome configurations of most of the complex-combinations which form a part of its complicated pedigreed history, including *flavens* · *curvans*.² We can state, however, that the chances are strong that *flavens* · ^h*Hookeri*, with its circle of 4 and 5 pairs, will have passed on ^h*Hookeri* complexes which were contaminated with *flavens* chromosomes, and that the resultant *Hookeri-curvae* did not contain pure ^h*Hookeri*. The chromosome configuration of *flavens* · *curvans* is said to be a circle of 12 and one pair (RENNER 1928). It is possible that the *flavens* which became associated with ^h*Hookeri* in this pedigree possessed one *curvans* chromosome, so that the ^h*Hookeri* complex which was received into *Hookeri-curva* from *flavens* · ^h*Hookeri* may have contained a *curvans* as well as *flavens* chromosomes. It is not surprising, therefore, to find that RENNER obtained some *Hookeri-curva* plants in this case with broad leaves (a *flavens* characteristic) and some with no red papillae on the stem (also a *flavens* characteristic).

I do not point out these facts because there is reason to believe that the chromosome configurations of the various *Hookeri-curvae* have been altered, with consequent alteration in the amount of splitting to be expected from them. On the contrary, there is reason to think that all of these *Hookeri-curvae*, however derived, may have had the same chromosome configuration. My purpose is rather to call attention to the possibilities of contamination when complexes are obtained from hybrids which have paired chromosomes, and the consequent necessity of knowing the chromosome configuration of hybrids before it is assumed that they are capable of passing on their complexes unaltered.

rigens · *flavens*

RENNER has pointed out (1925, p. 58) that *rigens* · *flavens* gives rise to progenies which show splitting in respect to the factors, *l_{et}*, *x* and *P*, *p*. In view of the fact that it possesses 4 independent chromosome groups, it is not surprising to find splitting in the progeny of this form.

² ((((*muricata* × *Lamarckiana*) *rigens* · *velans* × *muricata*) *velans* · *curvans* × *R-biennis*) *curvans* · *R-rubens* × *suaveolens*) *curvans* · *flavens* × *Hookeri*) *flavens* · ^h*Hookeri* × *r-muricata*.

^hgrandiflora B. ^hfranciscana Sh.

S. H. EMERSON (1929) has made a study of certain characters in crosses between *grandiflora* and *franciscana*, and has described the situation in F_2 in detail. He has pointed out that the F_2 shows a high degree of variation in respect to most of these characters, the evidence pointing to the presence of a number of independently segregating multiple factors. This author relates the presence of so many independent factors to the presence of independently paired chromosomes, as contrasted with the situation in many species crosses in *Oenothera*, where a large number of characteristics are inherited in blocs, and where the chromosomes are found in chains in F_1 . The correlation which this author suggests between the degree of pairing of chromosomes, and the degree of independence between factors, is the same as that suggested independently by CLELAND and OEHLKERS (1929).

My own F_2 culture of this cross was not nearly so extensive as that of EMERSON. It was grown in 1929 in order to test the degree of splitting present in the progeny of a hybrid possessing 6 independent chromosome groups. As far as the data go, they confirm EMERSON's findings entirely.

1929:15 *Oe. (grandiflora B. × franciscana Sh.) × s.*
(1925:6, pl. 3 × s.)

Germination was poor, only 35 out of 100 seeds producing seedlings. Of these, 34 were transferred to soil, but only 22 came up. Five of these had trouble with their seed coats, others were backward, with yellowish cotyledon bases. When it was time to set the plants out into the field, the situation was as follows:

4 large plants	{	one with broad, slightly toothed to entire, bright green leaves
		one with narrower, decidedly toothed, bright green leaves
		one with broad, decidedly toothed leaves, yellowish at base
		one with narrower, decidedly toothed leaves, yellowish at base
2 cripples, with forked leaves, and 2 growing points		
1 small green plant, with broad leaves		
4 fairly large, semi-chlorotic plants		
4 fairly large, chlorotic plants		
2 very small chlorotic plants.		

The remaining plants had died.

Ten of the healthiest plants were transferred to the field, of which the weakest one died. As the rest matured, no two of the plants were alike. In fact, it would be difficult to imagine a more varied assembly than was present in this small culture. Records were made in the case of several characteristics, as follows:

HABIT

Plants 1, 2, 5. Large and bushy, with well-developed central shoot and semi-erect side branches.

Plants 3, 8, 9. Rosette branches more spreading, nearly horizontal, relatively small. Central shoot prominent.

Plant 4. Rosette branches prostrate, central shoot reduced.

Plant 7. Rosette branches prostrate, about 1 m long. No central shoot. The whole plant lay flat on the ground.

Plant 6. Remained rosette.

FOLIAGE

Plant 1. Leaves wavy margined, slightly crinkled, dark green.

Plant 2. Leaves perfectly flat. Light green.

Plant 3. Leaves shallowly toothed, light green.

Plant 4. Leaves very narrow, troughed, dark green.

Plant 5. Leaves narrow but large, rather crinkled and wavy, dark green.

Plants 6, 7. Leaves broad, slightly crinkled, deeply toothed, light green. Others not recorded.

STEM

Diffuse color varied from deep red in plants 2 and 5 to scarcely a trace in plant 4. Color of tip red in all but 4. Red papillae present in all which flowered, the number varying with pubescence, which was heavy in some, sparse in others. In plant 3, hairs withered near the tip, so that stem tips became essentially glabrous.

BUDS

Plants 1, 2. Like *franciscana* in shape, size, color, but much hairier. Red papillae on ovary.

Plant 3. More like *grandiflora*, except for diffuse color, which was evenly distributed over the cone. Sepals sparsely hairy, tips long and delicate, no red papillae on the glabrous ovary.

Plant 4. Perfectly green, slender, more or less twisted, large, hairy, no red papillae.

Plant 5. Small, slender, with diffuse red on sepals and red papillae on ovary.

Plants 7, 8. Like plant 3, except for a few hairs, and red papillae on ovary.

FLOWER COLOR

Plant 5 had practically pure sulfur color. Plant 2 had light yellow flowers, approaching sulfur. In plant 4, certain stems bore sulfur-like flowers, and others bore deeper yellow. The rest of the plants had deep yellow flowers.

While the culture was of course too small to be of any value from the standpoint of genetic analysis, nevertheless it does serve the purpose for which it was grown, namely, to determine whether there was any segregation in the progeny of a hybrid with mostly paired chromosomes. There can be no doubt, both from EMERSON's data and from the above, that an abundance of segregation occurs during the maturation of the germ cells of this particular hybrid. This is in line with the chromosome cohesion hypothesis, inasmuch as the F_1 has 6 independent chromosome groups.

Since neither of the complexes making up the F_1 had a lethal, we would not expect the plants of the F_2 generation to breed true with respect to the presence of the circle of 4. Upon self-pollination, 50 percent of the progeny should have a circle of 4, and 50 percent should have 7 pairs,

barring further segmental interchange. Only 2 plants were examined cytologically (numbers 7 and 9). Both had a circle of 4, and 5 pairs. From plant 7, 16 complete cells were observed, and from 9, 18 cells.

albicans · ^h*franciscana*

This combination has a circle of 14. Both DAVIS (1916) and DE VRIES (1918) have described the F₂ as uniform, except that DAVIS has noted a considerable variation in flower size. Theoretically, there should be two types in F₂, a type like F₁ (*albicans* · ^h*franciscana*), and pure *franciscana*. HOEPPENER and RENNER (1929), however, have shown that *albicans* eggs are almost entirely suppressed by ^h*franciscana* in this combination; hence the only form produced as a rule in F₂ is *franciscana*. Apparently, the *franciscana* so formed is pure and uncontaminated by *albicans* genes, except possibly for flower size factors. The complexes are therefore passed to the germ cells essentially intact in F₁, as should be the case with a circle of 14 present. That flower size behaves independently of the chromosome configuration is already well known.

gaudens · ^h*franciscana*

This combination has a circle of 10 and 2 pairs. According to DE VRIES (1918) this hybrid splits into 2 main types, which are no doubt *gaudens* · ^h*franciscana* and ^h*franciscana* · ^h*franciscana*. This is to be expected in view of the alethal nature of ^h*franciscana*. The absence or near absence of splitting apart from this is in keeping with the fact that the hybrid has a circle of 10 chromosomes.

velans · ^h*franciscana*

This combination has a circle of 4 and 5 pairs. According to DE VRIES (1918) this hybrid breeds essentially true. This conclusion is apparently due to the genetical similarity between *velans* and ^h*franciscana*. RENNER has shown (1925, p. 97) that *velans* and ^h*Hookeri* are so closely related, that is, have so many genes in common, that it has been impossible as yet to detect with certainty the occurrence of exchange of factors between the two complexes. Since ^h*franciscana* is very similar to ^h*Hookeri*, it is probable that it too is very similar to *velans*, and hence one would not be surprised to find that an exchange of factors between ^h*franciscana* and *velans* had not been detected in spite of the presence of 6 independent chromosome groups.

This concludes the list of complex combinations dealt with in this paper, whose chromosome configurations and whose breeding behavior are both known. There remain to be considered three complex-combinations

whose breeding behaviors have been described but whose chromosome configurations have not as yet been determined. They are three of those whose configurations have been predicted as the result of data presented in this paper (see table 1 for predictions).

acuens · ^h*franciscana*

The predicted configuration is 2 circles of 4 and 3 pairs. This configuration would give the opportunity for a considerable degree of factor-exchange, the detection of which would depend upon the number of gene differences affecting visible characters. According to DE VRIES (1918), the F₂ of this hybrid splits into two types which repeat the two types found in F₁ of this cross (*grandiflora* de V. × *franciscana* de V.). It is impossible to understand, however, how a *truncata* can appear in the progeny of an *acuens* · ^h*franciscana*. Since DE VRIES' observations were made before the concept of complex-heterozygosis afforded a key to *Oenothera* problems, and since his results are at variance with the results obtained by CLELAND and OEHLKERS (1930) for the corresponding *Hookeri* cross, his observations should be checked. The corresponding *acuens* · ^h*Hookeri* yields a progeny which splits independently in respect to at least 4 factors, and one would therefore expect to find splitting in the progeny of *acuens* · ^h*franciscana* as well.

truncans · ^h*franciscana*

This combination should have a circle of 14 as its configuration. DE VRIES (1918) has reported that this form breeds true in the main, though with variations in minor characters, including flower size. His failure to obtain *franciscana* segregates in F₂ is not according to expectation, inasmuch as the corresponding *truncans* · ^h*Hookeri* yields them in large numbers (CLELAND and OEHLKERS 1930, p. 40). The presence of variation in characters other than flower size is also unexpected, since the progeny of *truncans* · ^h*Hookeri* shows no factorial splitting except in flower size. Whether there is actually as great a divergence in the behavior of ^h*franciscana* and ^h*Hookeri* when combined with *truncans* as appears from a comparison of DE VRIES' results with those of CLELAND and OEHLKERS is doubtful. With the probable exception of flower size, we should expect the progeny of *truncans* · ^h*franciscana* to show no factorial splitting.

rubens · ^h*franciscana*

This combination should have a circle of 10 and 2 pairs. DE VRIES reports (1918) the splitting into two main types in F₂, with minor variations. DAVIS also found two main classes (1916). HOEPPENER and RENNER (1929,

p. 57) state that exchange may occur independently from one complex to the other in respect to 3 factors, C_o , R and S_p . With only 2 independent pairs of chromosomes, the presence of 3 factor pairs independent of the complex is not what one would expect. However, one of these is flower size, which is the one factor which is known to act independently of chromosome behavior. Whenever we have found one more linkage group than there were chromosome groups, C_o or an equivalent factor for flower size is one of the independent factors involved.

In summary, it may be said that, except for splitting in flower size, the F_2 of hybrids whose breeding behavior has been determined subsequent to the development of the concept of complex-heterozygosity behave in a manner which conforms to the type of chromosome configuration possessed by F_1 . There is not so close an agreement in the case of *acuens* · *^hfranciscana* and *truncans* · *^hfranciscana*, whose breeding behavior was studied without the benefit of this concept. However, the discrepancy between the breeding behavior of these forms, and that of the closely allied *acuens* · *^hHookeri* and *truncans* · *^hHookeri*, together with the fact that the latter show a behavior in conformity with the chromosome configurations which they possess, suggests that a re-study of the two *franciscana* hybrids will disclose a behavior which is also in agreement with chromosome configuration.

In general, therefore, the data derived from a study of certain of the forms included in this paper are in support of the theory that chromosome cohesion in *Oenothera* is responsible for the linking of genes which reside in different chromosomes, and consequently for the existence of the RENNER complex.

The cause of chromosome cohesion

When *Oenothera* species are crossed, the hybrids which are formed have characteristic and constant chromosome configurations in meiosis. In some cases all of the chromosomes are united end to end to form circles; in others some chromosomes are thus united, and some are paired. If the association of chromosomes in meiosis is a result of the association of homologous parts of chromosomes, then it must be that not all the chromosomes contributed by the two parents of a circle-bearing hybrid have had the same arrangement of homologous parts (see HÅKANSSON 1928, 1930, DARLINGTON 1929, 1931, EMERSON 1931, CLELAND and BLAKESLEE 1930, 1931). Portions of a given chromosome from one parent must have been homologous with parts of 2 separate chromosomes from the other parent; else this chromosome would not have united at one end to one chromosome and at the other end to another chromosome from the other parent.

If then we are ready to assume that the association of chromosomes in meiosis is a result of the association of homologous parts, we are forced to the conclusion that homologous parts are differently distributed among the chromosomes in the various genetic complexes in *Oenothera*, at least as far as the *ends* of the chromosomes are concerned (we do not know as yet how far back from the ends of the chromosomes these differences in arrangement go). Each complex has its own characteristic and specific arrangement of homologous end parts, and thus must give with every other complex a definite and predictable chromosome configuration. The fact, however, that each complex has its own specific arrangement of ends, which is different from that of other complexes, can be understood only on the assumption of an antecedent occurrence of mutual exchanges of segments between originally homologous chromosomes. At least such a process of interchange is a logical necessity unless we are prepared to argue for a polyphyletic origin of the genus, or for the doctrine of Special Creation. DARLINGTON's criticism (1931, p. 467), therefore, of CLELAND and BLAKESLEE's predictions as to chromosome behavior, based (according to these authors) upon the segmental interchange theory, on the ground that their arguments bore only on the question of specificity and not upon the question of segmental interchange, is invalid, for such specificity of end arrangement as is proved by successful prediction of chromosome configurations could have come about only through segmental interchange, and, consequently, proof of specificity constitutes at the same time proof of segmental interchange.

CLELAND and BLAKESLEE (1930, 1931) were able to show in the case of 4 hybrids that the chromosome configuration possessed by each of them was the only one possible on the basis of the segmental interchange theory. This constituted an actual proof of the validity of the theory as applied to *Oenothera*. It would be well, however, to considerably amplify this type of evidence before the theory is considered as definitely established in the case of *Oenothera*. Additional evidence is furnished in connection with certain of the forms dealt with in this paper.

It has been shown above that both ^h*franciscana* de V. and ^h*franciscana* Sh. give 7 pairs in association with ^h*Hookeri* de V. This means, on the basis of the theory, that the chromosome sets comprising these complexes are identical with respect to the way in which end segments are distributed. Each chromosome of ^h*franciscana* has the same ends as a corresponding chromosome of ^h*Hookeri*, and therefore will pair with it; and the formula for the end arrangement of ^h*franciscana* will be the same as that for ^h*Hook-*

eri (^h*Hookeri* has been given the formula 1·2 3·4 5·6 7·8 9·10 11·12 13·14). Since, then, ^h*franciscana* has the same chromosomes as ^h*Hookeri*, with respect to end arrangement, it should give the same configuration as ^h*Hookeri*, when brought into association with a third complex. It is therefore possible to make the following *general* prediction with reference to the chromosome configurations to be expected in hybrids of *franciscana*, assuming that homologous ends unite in diakinesis: *hybrids involving ^hfranciscana deV. and ^hfranciscana Sh. will be found to have identically the same chromosome configurations as corresponding hybrids involving ^hHookeri deV.* On the strength of this general prediction, 11 specific predictions with regard to particular *franciscana* hybrids may be made at the present time, inasmuch as the configurations of that many ^h*Hookeri* crosses are now known, and the corresponding ^h*franciscana* crosses are certain to have the same configurations as these. These specific predictions are listed in table 1.

TABLE 1

Chromosome configurations, predicted and actual, in hybrids of franciscana Sh. and franciscana deV.

HYBRID	PREDICTED CONFIGURATION	ACTUAL CONFIGURATION
<i>acuens</i> · ^h <i>franciscana</i>	2⊙s of 4, 3 pairs	?
<i>albicans</i> · ^h <i>franciscana</i>	⊙ 14	⊙ 14
<i>excellens</i> · ^h <i>franciscana</i>	⊙ 4, 5 pairs	?
<i>flavens</i> · ^h <i>franciscana</i>	⊙ 4, 5 pairs	⊙ 4, 5 pairs
<i>gaudens</i> · ^h <i>franciscana</i>	⊙ 10, 2 pairs	⊙ 10, 2 pairs
<i>rigens</i> · ^h <i>franciscana</i>	⊙ 6, 4 pairs	⊙ 6, 4 pairs
<i>rubens</i> · ^h <i>franciscana</i>	⊙ 10, 2 pairs	?
<i>truncans</i> · ^h <i>franciscana</i>	⊙ 14	?
<i>velans</i> · ^h <i>franciscana</i>	⊙ 4, 5 pairs	⊙ 4, 5 pairs
<i>curvans</i> · ^h <i>franciscana</i>	⊙ 6, ⊙ 8	⊙ 6, ⊙ 8
^h <i>franciscana</i> · <i>punctulans</i>	⊙ 10, 2 pairs	?

It has not been possible as yet, nor perhaps is it necessary, to test all of the predictions contained in this table. But 6 hybrids in the list have been examined cytologically, as recorded in the descriptive section of this paper. In all 6 cases, the configuration actually possessed by the hybrid is the one which was predicted. (It may be well to call attention to the fact that these were all real predictions. The first crosses examined cytologically in the fall of 1930 were those between *franciscana* and *Hookeri*, which were all found to have 7 pairs. The above predictions were then made. All of the tests were made subsequent to the predictions so that the results constitute an actual confirmation of *bona fide* predictions.)

These successful predictions, taken together with the 4 previously reported by CLELAND and BLAKESLEE, furnish a highly significant body of

proof for the segmental interchange theory as applied to *Oenothera*. So far no failures but rather 10 consecutive successes have resulted from attempts to predict chromosome configurations on the basis of this theory. Furthermore, STURTEVANT and EMERSON have had a like degree of success in making predictions (private correspondence). When we consider that definitive predictions as to chromosome configuration cannot be made except on the basis of segmental interchange, such uniform success in prediction makes it possible to accept the segmental interchange theory, as applied to *Oenothera*, with a considerable degree of assurance.

SUMMARY

Field and cytological data are presented bearing upon a number of hybrids of the following species or strains of *Oenothera*: *muricata*, *Hookeri* deV., *r-Lamarckiana*, *suaveolens*, *grandiflora* deV., *franciscana* deV., and *franciscana* Sh. These data are discussed in relation to two problems of *Oenothera* research:

The genetical effect of chromosome cohesion in Oenothera

The breeding behavior of a number of hybrids discussed in this paper is known. In all of these in which genetical behavior has been determined subsequent to the formulation of the concept of complex-heterozygosity the degree of splitting in F_2 is comparable to the number of independent chromosome groups in F_1 . Hybrids with few chromosome groups give little splitting in F_2 , and *vice versa*. Flower size continues to be an exception to the rule, inasmuch as some of these hybrids show splitting in respect to this factor in spite of the presence of a chromosome arrangement which would seem to preclude it. In 2 cases where breeding behavior was described prior to the formulation of the concept of complex-heterozygosity there appears to be less of a correlation between genetical and cytological behavior. Such cases should be re-investigated. On the whole, however, the correspondence between genetical and cytological behavior in the hybrids considered in this paper is such as to furnish positive evidence in favor of the chromosome cohesion theory of extensive genetical linkage in *Oenothera*.

The cause of chromosome cohesion

It has been possible successfully to predict in advance the chromosome configuration of 6 of the hybrids under consideration, reasoning upon the basis of segmental interchange. This, together with previous results, makes a total of 10 published cases in which definite predictions have been made.

As yet no failures have been recorded. The uniformity with which successful predictions have been made, together with the impossibility of making definitive predictions on any other basis, makes it strongly probable that circle formation in *Oenothera* has come about through the process of segmental interchange.

Note: Since completion of this manuscript, three papers by STURTEVANT and EMERSON have come to hand. In these further predictions will be found, some of which have been verified by the authors. The evidence for segmental interchange is thus augmented still further. These papers are listed below.

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