AN AMPHIDIPLOID IN THE F_1 GENERATION FROM THE CROSS OENOTHERA FRANCISCANA×OENOTHERA BIENNIS, AND ITS PROGENY*

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Received January 19, 1943

CERTAIN peculiarities of the cross *Oenothera franciscana* Bartlett×*Oenothera biennis* L. led me to grow in recent years some large F_1 families in one of which there appeared a tetraploid plant the cytology and genetical behavior of which showed it to be an amphidiploid. This plant and its progeny will be the subject of this paper.

The cross *franciscana*×*biennis* from parents out of long inbred lines produces in the F_1 a progeny chiefly of light green, weak seedlings only a few of which live to develop small pale rosettes and these unable to live in the field. This was first recorded by DAVIS (1914, p. 189) and has been noted by later workers who have grown small families of the cross. But in contrast to the large progeny of light green, weak plants that die, there is a small group of dark green, strong seedlings which produce vigorous plants that come to flower in the field. The contrast between young rosettes of about the same age is shown in the text figure 1; above are the parent types, *franciscana* at the left and *biennis* at the right; below are the two types of F_1 hybrids.

Combined data from three large cultures (35.71, 36.71, 36.77) with a germination of about 95 percent gave 3672 pale green seedlings and rosettes almost all of which died rapidly in pans and pots and none of which survived in the field or when given special attention in the greenhouse. There were 44 plants, dark green and strong, all of which matured, and among these were three triploids and the amphidiploid.

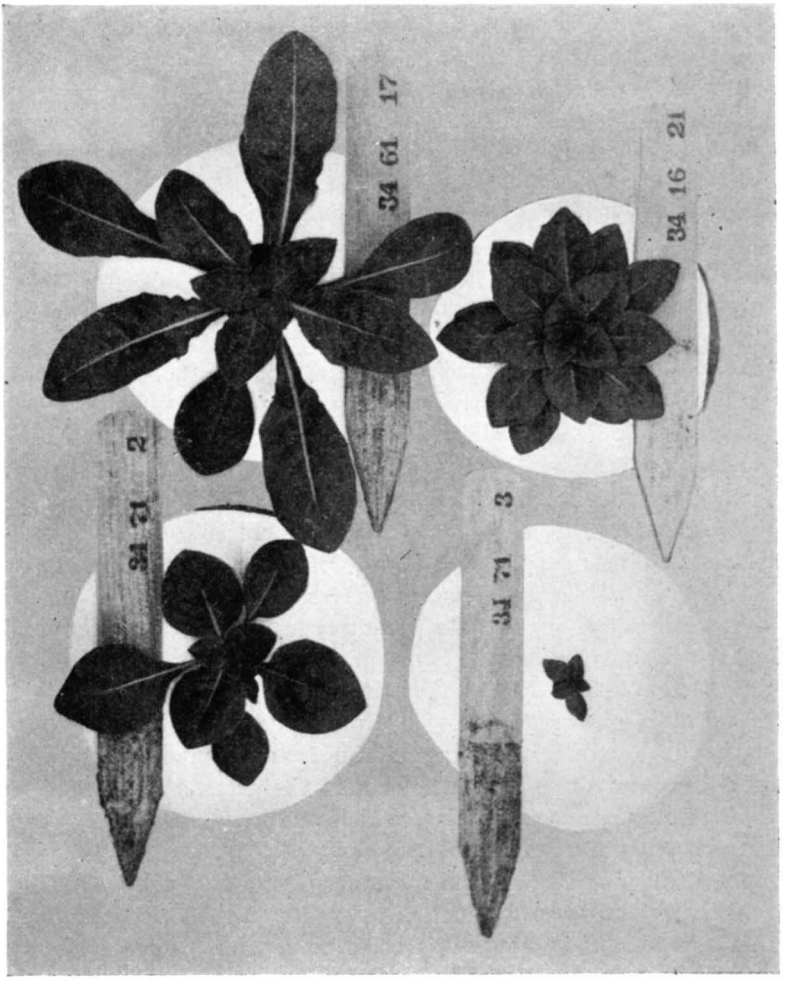
The three triploids differed from the other plants of the dark green type in greater vigor, larger leaves, absence of red spots on the rosette leaves, and in green bud cones. Of the pollen scarcely 10 percent of the grains seemed well developed, and these were probably infertile, since the plants produced no seed after numerous selfings, although forming large capsules when open pollinated. Cytological studies of meiosis in pollen mother cells of two plants showed that irregular distribution of the 21 chromosomes (10–11, 9–12, 8–13) characteristic of Oenothera triploids, and also showed frequent elimination of chromosomes through lagging on the spindle.

The amphidiploid was distinguished markedly from the other dark green plants in the following respects:

	Amphidiploid	Dark green plants
Mature rosette	5 dm broad. Leaves 25 cm long, 7–8 cm broad, much thicker.	3.5 dm broad. Leaves 18 cm long, 5–6 cm broad.

* The cost of the accompanying halftone illustrations is borne by the Galton and Mendel Memorial Fund.

[1] Cytological studies on Oenothera. VI. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 712.



Text figure 1. *franciscana* upper left. *biennis* upper right.
Below, the two types of F_1 hybrids.

	Amphidiploid	Dark green plants
Mature plant	Central shoot 4.5 dm. Leaves at base 15 cm long, 5.5 cm broad, much thicker.	Central shoot 7–9 dm. Leaves at base 13 cm long, 4.5 cm broad.
Petals	27 mm long.	23 mm long.
Pollen grains	30 percent good, larger, 4-lobed, an occasional 3-lobed grain.	35–50 percent good, 3-lobed, an occasional 4-lobed grain.
Capsules	22–25 mm long, much stouter.	18–20 mm long, medium stout.
Chromosomes	N = 14.	N = 7.

The amphidiploid then differs from the dark green type in having larger and thicker leaves, larger flowers, longer and stouter capsules, and larger pollen grains generally 4-lobed.

The seeds were germinated in Petri dishes over wet filter paper. The cytological material was fixed in Karpechenko-Navashin with three changes during eight hours, washed over night, carried through a long series of alcohols, and stained with crystal violet after the method of Gram.

CYTOLOGY OF THE DARK GREEN DIPLOID PLANTS, F_1 FRANCISCANA × BIENNIS

Studies on two diploid plants (35.71–18 and 35.71–29) were in essential agreement with the prophecy of CLELAND (1932, p. 596) that the dark green hybrids from the cross *franciscana* × *biennis* would present the chromosomes at diakinesis in the configuration of a circle of ten and two pairs. I have not observed any closed circles of ten chromosomes but long chains are characteristic, sometimes a single one of ten chromosomes (fig. 1), but more frequently two or more chains of smaller numbers as in figure 2. The two pairs are very clear. The chains may still be found at metaphase of the first meiotic division. There are numerous irregularities of chromosome distribution during meiosis, responsible for at least part of the high degree of pollen abortion.

CYTOLOGY OF THE AMPHIDIPLOID

On the assumption that the amphidiploid arose from the diploid through somatic doubling of the chromosomes (see DISCUSSION) the 20 daughter chromosomes derived from the circle of ten or from equivalent chains would naturally tend to pair at diakinesis. However, where so large a number is concerned a failure of mates to find one another would be expected to lead to some groupings as trisomes, and longer chains or circles might be present. The four chromosomes derived from the doubling of each of the two pairs might give pairs at diakinesis or they might associate end to end to form circles or chains of four chromosomes. Therefore, apart from irregularities leading to trisomes, there might appear in the amphidiploid at diakinesis 14 pairs, or pairs and circles or chains of four or more, or no pairs but only circles or chains of four or more.

Figure 3 shows an example of diakinesis in which pairing seems to be proceeding in a regular manner, whereas in figure 4 chain arrangements are present that are confusing and might result in irregularities of meiosis. An illustration of all pairing at metaphase of the first meiotic division is given in figure 5; examples of such regularity were not easily found. Much more frequent were irregularities such as are shown in figure 6 where chains of three chromosomes are shown. Sometimes chains of four will be present as in figure 7, and such chains might consist of the four derivatives from the doubling of the pairs in the hybrid, or they might arise from the derivatives of the doubling of any two adjacent chromosomes in a chain.

Observations on the split chromosomes of interkinesis in the amphidiploid quickly established irregularities of chromosome count and distribution in the first meiotic division. Examples of regular distribution are not common. Segregation of chromosomes 15–13 were found. There is much lagging of chromosomes during the first division with the result that nuclei with 13 chromosomes are frequently formed (fig. 8, 9). In figure 9 all the chromosomes are accounted

for by a distribution of 13 with one, and 11 associated with three. It became established through plants in the generation from the amphidiploid selfed that some at least of the gametes with 13 chromosomes are functional. The irregularities of chromosome distribution must play a large part in the explanation of the high degree of pollen abortion in the amphidiploid.

In summary it should be emphasized that this amphidiploid did not present a settled behavior of all pairing on the part of the chromosomes at diakinesis. On the contrary, there was much irregularity in the process of chromosome segregation during meiosis. Accounts of amphidiploids have frequently assumed that these plants even from hybrids would breed true because the double set of chromosomes would permit a regular pairing between homologues. It will be noted that here is an amphidiploid Oenothera hybrid in which the pairing is far from regular with the result that the plant does not breed true, as will appear in the accounts of later generations.

A GENERATION FROM THE SELFED AMPHIDIPLOID

Here follows the history of a progeny from the selfed amphidiploid (culture 37.103). There were 355 seedlings from 426 seeds (three capsules), a germination of 83.3 percent. Seedlings and young rosettes were vigorous and presented a wide range in the width of the thick leaves. A representative group of 175 plants was set in the field. Mature rosettes followed the lead of younger, ranging from large with leaves about 20 cm long and 8–10 cm broad to small with leaves about 10 cm long and 5 cm broad. There were 41 large-leaved rosettes, 16 small-leaved, and 46 rosettes with leaves intermediate in size; 72 rosettes died in the field.

Of the 103 rosettes that lived only 31 produced flowering shoots. These plants were grouped in three classes.

1. Large flowers. These were represented by 11 plants from large rosettes, petals 30–35 mm long. They were larger-flowered and generally larger plants than the amphidiploid parent.

2. Medium-sized flowers. These included 18 plants from rosettes of medium size, petals 20–25 mm long. Most of the plants were similar to the amphidiploid.

3. Small flowers. There were two plants from medium-sized rosettes with petals 10–15 mm long and short capsules 14–16 mm long.

None of the small-leaved rosettes produced flowering shoots. Good pollen was usually 4-lobed, but some grains were 3- or 5-lobed. In certain plants the good pollen was as high as 90 to 95 percent of the output, but there were generally many shriveled grains present and good pollen in much smaller proportions, ranging from 10 to 80 percent.

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 (N.A. 1.5) primary magnification 120, in combination with the compensating ocular K20. The figures are reproduced as magnified at stage level—that is, 2400 diameters.

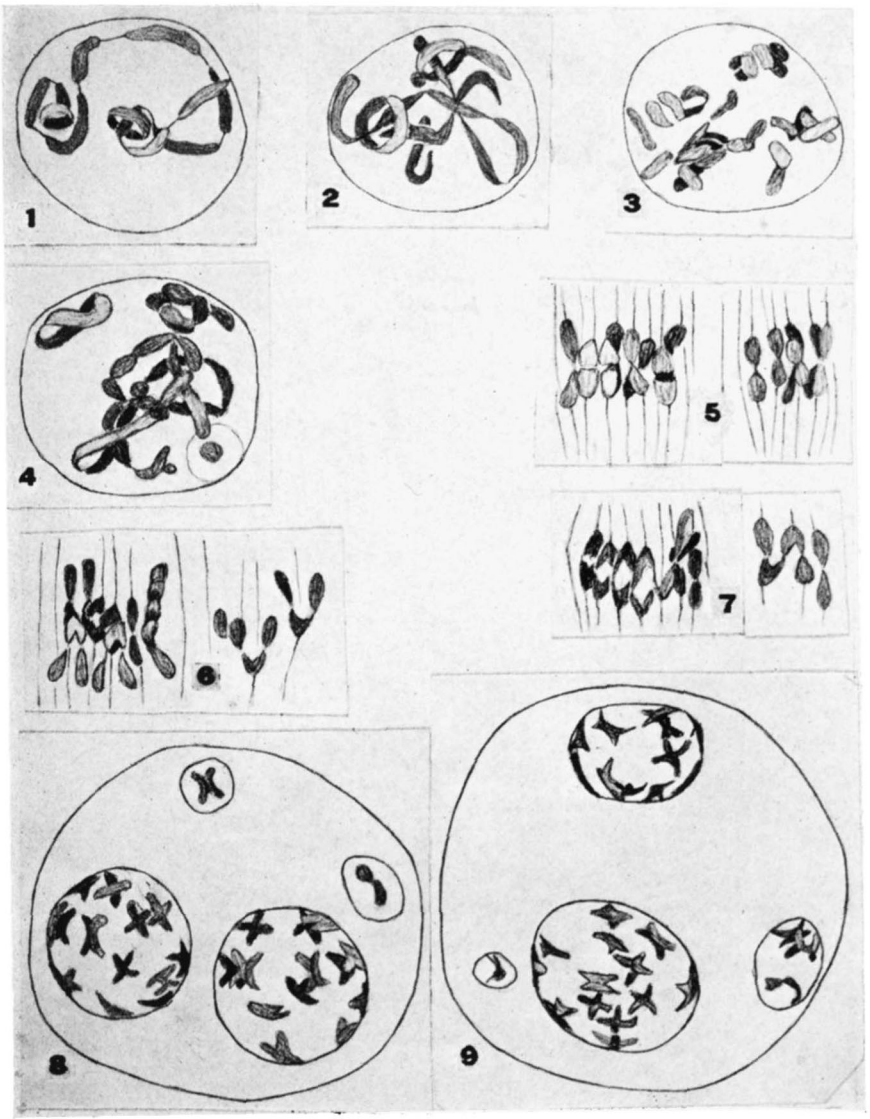


PLATE 1

FIGURE 1.—F_1 *Oenothera franciscana*×*O. biennis*. Diakinesis, chain of ten chromosomes and two pairs.

FIGURE 2.—F_1 *franciscana*×*biennis*. Diakinesis, shorter chains and two unpaired chromosomes, two pairs.

FIGURE 3.—Amphidiploid. Diakinesis, regular pairing apparently in progress.

FIGURE 4.—Amphidiploid. Diakinesis, chain arrangements present.

FIGURE 5.—Amphidiploid. First meiotic division, chromosomes all paired.

FIGURE 6.—Amphidiploid. First meiotic division, chains of three chromosomes shown.

FIGURE 7.—Amphidiploid. First meiotic division, chain of four chromosomes present.

FIGURE 8.—Amphidiploid. Interkinesis, two nuclei with 13 chromosomes the result of lagging of one pair.

FIGURE 9.—Amphidiploid. Interkinesis, irregular distribution to give two large nuclei with 13 and 11 chromosomes associated with two small nuclei of one and three chromosomes, respectively.

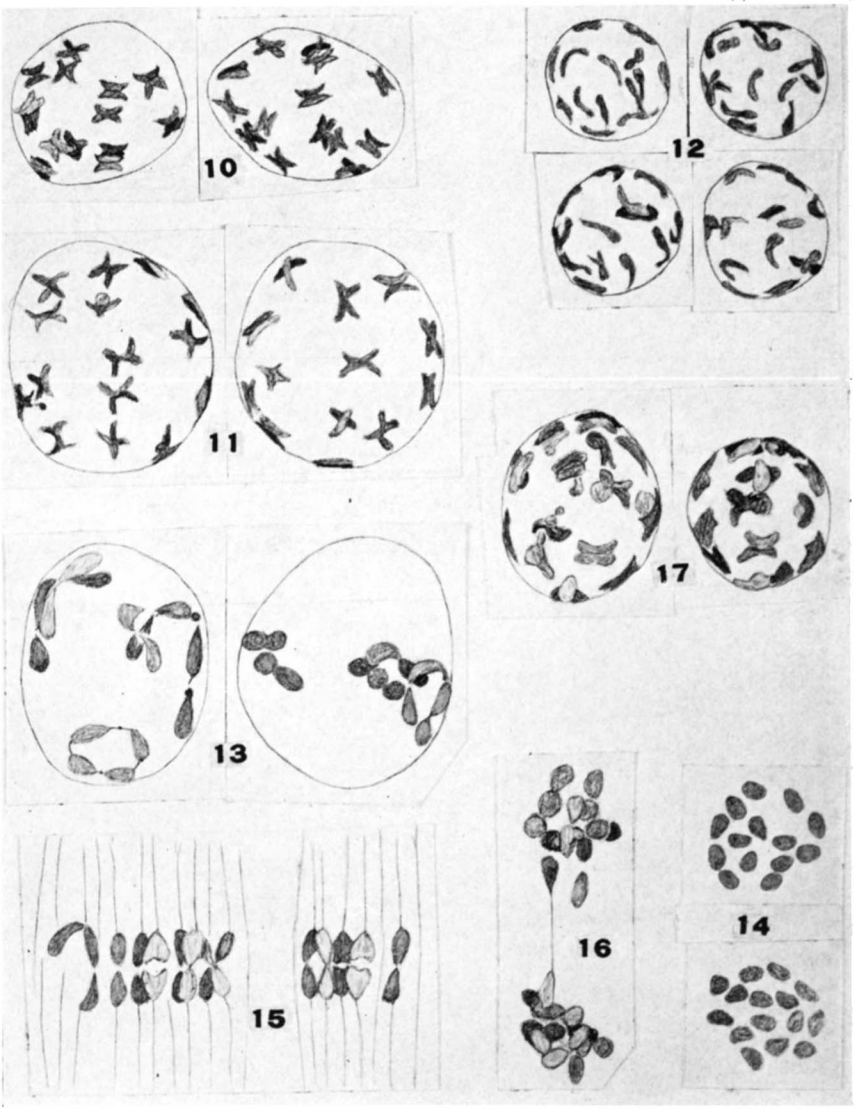


PLATE 2

FIGURE 10.—Plant No. 11. Interkinesis, the 28 chromosomes segregated 14–14.

FIGURE 11.—Plant No. 11. Interkinesis, the 28 chromosomes segregated 15–13.

FIGURE 12.—Plant No. 11. Nuclei from a tetrad, daughter chromosomes distributed by second meiotic division 14–14 and 15–13.

FIGURE 13.—Plant No. 34. Diakinesis, the 28 chromosomes in pairs, trisomes, and chains of four.

FIGURE 14.—Plant No. 45. The 29 split chromosomes at metaphase of the second meiotic division following a segregation 15–14.

FIGURE 15.—Plant No. 155. The 27 chromosomes at metaphase of the first meiotic division, 12 pairs and a trisome.

FIGURE 16.—Plant No. 155. Anaphase of first meiotic division, the unpaired chromosome backward in reaching a pole.

FIGURE 17.—Plant No. 155. Interkinesis, the 27 chromosomes segregated 14–13.

From this assemblage four plants were selected for selfing and for cytological studies. The plants were Nos. 11, 34, 45, and 155, and the findings were as below:

Plant No. 11. Large Flowers

Plant No. 11 with 28 chromosomes had large flowers (petals 30 mm), a large rosette, pollen 90 percent good, pollen grains 4-lobed with very few 3-lobed, germination from 379 seeds 31.1 percent.

The count of 28 chromosomes in Plant No. 11 was made in the two nuclei of interkinesis (fig. 10) from 20 cells, in five examples of the second division, and from two tetrads. There was much irregularity of distribution expressed in trisomes and chains of four in the first division, the former resulting in the formation of nuclei with 13 chromosomes either through non-disjunction in the distribution 13–15 (fig. 11), or by the lagging of one chromosome. Stages of interkinesis with nuclei showing 13 chromosomes were noted in 19 cells. The distribution 15–13 was found in four examples of interkinesis, in one second division, and in two tetrads (fig. 12). Rarely a nucleus was found with 12 chromosomes.

A family (culture 38.51) was grown from seeds of plant No. 11 selfed. The 118 young rosettes showed a wide range from large broad-leaved to small narrow-leaved plants. The latter were weak, and only 93 rosettes could be set in the field. Only 40 plants produced flowering shoots, 33 large-flowered and similar to the parent, and seven with medium-sized flowers. The genetical behavior of the plant was very similar to that of its parent amphidiploid. Therefore, Plant No. 11, like its parent, proved to be very far from a stable amphidiploid.

Plant No. 34. Medium-sized Flowers

Plant No. 34, also with 28 chromosomes, had medium-sized flowers (petals 23 mm), large rosette, pollen 80 percent good, pollen grains 4-lobed with few 3-lobed, germination from 481 seeds 45.3 percent.

The presence of 28 chromosomes was established by counts from 15 cells with the two nuclei in interkinesis, from some polar views of second division, and from the nuclei of two tetrads. As in plant No. 11, there was much irregularity at diakinesis expressed in trisomes and chains of four (fig. 13), although surprisingly large numbers of pairs emerged at metaphase of the first division. Non-disjunction and lagging gave numerous examples of nuclei with 13 chromosomes.

The generation grown from plant No. 34 selfed (culture 38.52) came through very well and showed much less variation than that from plant No. 11. There were set in the field 211 plants out of 218 seedlings, and of these 187 plants flowered. The mature rosettes were fairly uniform and of medium size. The flowers ranged from medium size to large. This rather uniform progeny (except for flower size) was much more like that to be expected from an amphidiploid than the generations from the other plants studied.

Plant No. 45. Dwarf, Seed Sterile

Plant No. 45 was a dwarf with 29 chromosomes, central shoot 3 dm high, flowers medium-sized (petals 20 mm), sepals very hairy, pollen 40 percent good, pollen grains 4-lobed with few 3- or 5-lobed, no germination from 90 seeds.

The count of 29 chromosomes was made from eight examples of interkinesis showing the two nuclei with 14 and 15 chromosomes, respectively, from two cells with nuclei in metaphase of the second division (fig. 14) and in three tetrads. The distribution of the chromosomes at interkinesis was usually 14–15, but lagging frequently gave nuclei with 13 chromosomes. Seven other plants in the culture were similar in appearance to Plant No. 45.

Numerous self-pollinations were made, but only one small capsule developed with 90 seeds that failed to germinate. The seeds although fair-sized had very little contents.

Plant No. 155. Small Flowers

This 27 chromosome plant was characterized by small flowers (petals 10 mm), small leaves, medium-sized rosettes, pollen 60 percent good, pollen grains about half 4-lobed and half 3-lobed with a few 5-lobed, capsules small (14–16 mm long), germination from 124 seeds 59.9 percent.

The chromosome count of 27 was determined in two examples of first division (fig. 15, 16) in 14 nuclei in interkinesis (fig. 17) in two examples of second division and in three tetrads. The odd or unpaired chromosome is sometimes alone at the side of the equatorial plate in the first division, or it may be associated with a pair to form a trisome (fig. 15). It frequently moves less rapidly to the pole of the spindle (fig. 16), and sometimes fails to be included in a daughter nucleus. The distribution 13–14 (fig. 17) was common. A few nuclei of interkinesis were noted with 12 and 15 chromosomes the results of nondisjunction.

The plant set small capsules with few seeds and did not hold the capsules well. From 18 selfings only four capsules were collected producing a total of 124 seeds. Of these seeds 63 proved fertile and gave 60 rosettes that were set in the field. The rosettes at maturity were fairly uniform and of medium size, but the mature plants fell into groups distinguished by flower, leaf, and capsule size. There were nine plants like the parent, with small flowers, leaves, and capsules; 30 plants with medium-sized flowers and large capsules; nine plants with large flowers, large leaves, and large capsules; nine rosettes which failed to produce flowering shoots; three plants that died.

This is a segregation such as one might expect from the 27 chromosome parent. The small-flowered plants probably repeated the combination 13–14 of the parent and the larger plants with large flowers, leaves, and capsules, the 14–14 combination of the amphidiploid.

Some of the early studies on amphidiploids, analyzed by WINGE (1932), led to a high degree of confidence in their stability and fertility as "constant species hybrids." But as more examples have been investigated it has become evident that irregularities of chromosome distribution at meiosis are common

and that the pairing of sister chromosomes (following somatic doubling) may not take place as regularly as might be expected. Other conditions, discussed by GOODSPEED and BRADLEY (1942), also lead to inconstancy of amphidiploids, but irregularities of chromosome distribution probably cause the greatest disturbance. The amphidiploid from *franciscana*×*biennis* illustrates well that inconstancy which goes with irregularities in chromosome associations and distribution expressed in aneuploidy and forms of sterility.

DISCUSSION

We are dealing with a tetraploid in the F_1 of the cross *franciscana*×*biennis*, and there would seem to be only two possible ways by which it might have arisen. First, and much more probable, a doubling of the two parental sets of chromosomes may have taken place in the zygote or young embryo through the failure of one or more mitoses to effect the distribution of daughter chromosomes, thus establishing reorganized somatic nuclei with double sets of chromosomes. Second, possible but improbable, a chance diploid gamete of *biennis* might have met a chance diploid gamete of *franciscana*. The first suggestion follows the views of GATES (1909, 1911) on Oenothera and the hypothesis of WINGE (1932) on "constant species hybrids" for which much supporting evidence is known. The second possibility is unlikely, since triploids which show the presence and functioning of diploid gametes are rare in pure line cultures of *biennis* and *franciscana*. My observations on the origin of an amphidiploid in the F_1 of a cross add to the findings of RENNER (1933) who has reported five highly fertile and fairly constant tetraploids directly from crosses.

When amphidiploids come from a parent with chromosome complexes of one or more circles, it becomes a matter of interest to know to what extent such chain associations may appear at diakinesis. The *franciscana* parent of this amphidiploid was of a race with all pairing chromosomes, but the *biennis* parent had the complex of two circles of six and eight chromosomes, respectively. The dark green hybrid *franciscana*×*biennis* presents at diakinesis a chain or circle of ten chromosomes and two pairs. Although the material studied was limited, large circles were not found at diakinesis in the amphidiploid, at best only chains of three and four chromosomes.

SEITZ (1935) has published a large body of observations on chromosome arrangements in the prophases of the first meiotic division in tetraploids from *franciscana*, *biennis*, and *Lamarckiana*. The observed associations are shown in tables for a large number of nuclei. Important are his findings that the largest circles and chains contain no more chromosomes than would be expected from the configurations in the parent plants. His material of *franciscana gigas* was from a race of *franciscana* with a circle of four chromosomes and five pairs, and SEITZ found in the tetraploid circles or chains of eight chromosomes but never more than one in a single nucleus.

The material of *biennis gigas* studied by SEITZ presented most of the chromosomes in pairs or quadrivalents. However, since the chromosomes of *biennis* are in circles or chains of six and eight, such might occur in the tetraploid, and they were found. The persistence of some groups of six and eight was remark-

able, one nucleus showing two of each. Another nucleus had two chains of 12 and two pairs; this condition might arise through breaks in the circles at the same point and the union of free ends, accompanied by the splitting off of two pairs.

SEITZ reports for *Lamarckiana gigas* at diakinesis numerous pairs and less frequently quadrivalents in chains and circles, together with occasional single chromosomes, trisomes, and groups of more than four. Since the chromosome configuration of *Lamarckiana* is a circle of 12 and one pair, larger circles and chains than four might be expected in *gigas*, but they were rarely found. The failure to recover anything resembling the circle of 12 in *Lamarckiana* is an important point in this account. The larger circles of diploids are maintained with greater difficulty in tetraploid derivatives. The greater the number of chromosomes in the convolutions of a chain the greater will be the probability of breaks when related chromosomes in two chains find themselves in favorable positions to pair in diakinesis. Irregularities of chromosome distribution at meiosis leading to high pollen sterility have been reported for *Lamarckiana gigas* from the earliest accounts of its cytology (GATES 1909, 1911; DAVIS 1911; HOEPPENER and RENNER 1929). BOEDIJN (1924) described a *gigas-lata* with 29 chromosomes and this must assume the functioning of a 14+1 gamete.

It is well known that *Lamarckiana gigas* throws a varied progeny: narrower-leaved and smaller-flowered forms, some of them dwarfs, besides the broad-leaved large-flowered type that carries the line. There is thus close parallelism in both cytological and genetical behavior between *Lamarckiana gigas* and the amphidiploid described in this paper. But this parallelism would be the same whether *gigas* arose by somatic doubling or through the fusion of suitable diploid gametes. CLELAND (1929, p. 138) has reported tetraploid pollen mother cells in a loculus of *Lamarckiana* all in heterotypic metaphase; from such cells would be expected diploid pollen with two sets of *Lamarckiana* chromosomes. The triploids (semi-gigas) that occasionally appear in lines of *Lamarckiana* prove the functioning of diploid gametes, and extensive breeding from such triploids (highly sterile) out of *Lamarckiana* accompanied by detailed cytological studies might establish the origin of a tetraploid through the fusion of diploid gametes. It seems most improbable that dispermy has played an important part, if any, in the origin of triploids in Oenothera (DAVIS 1933, p. 294). However, the possibility that the original *gigas* plant came from *Lamarckiana* through somatic doubling can never be ruled out.

The position of GATES (1909) who early held that *Lamarckiana gigas* arose through somatic doubling of the chromosomes of *Lamarckiana* (a structural hybrid) has support in the findings of RENNER (1933) of five highly fertile and fairly constant tetraploids among the progeny of direct crosses, and in the appearance of this amphidiploid in the F_1 from the cross *franciscana*×*biennis*. These tetraploids are all from diploid ancestry. But this does not weaken as an hypothesis the position of STOMPS (1912) who proposed the view that the union of suitable diploid gametes from triploids would give tetraploids. Since triploids (*semi-gigas*) are known from many lines of Oenothera, it is neces-

sary only to secure sufficient genetical and cytological evidence to place this hypothesis in a strong position.

Such evidence for the fusion of diploid gametes came through the history of a tetraploid *franciscana* by way of a triploid in the F_2 of the cross *franciscana*×*franciscana sulfurea nana* (DAVIS 1933). This is not the place to repeat the details of this genetical history and the cytological behavior that accompanied it, but the following important points may be emphasized because of a postscript to RENNER'S paper of 1933. The 21 chromosomes of the triploid were believed to be in three homologous sets because they grouped themselves at diakinesis in trisomes. Segregation of 7–14 chromosomes (one set and two sets) took place; much irregularity in association and distribution of the chromosomes was responsible for a high degree of pollen sterility. The triploid gave a progeny of 42 *franciscana* plants (14 chromosomes), 44 various dwarfs, and three thick-leaved tall plants (probably all tetraploids), one of which was established to be a tetraploid. The evidence is very strong that the 42 *franciscana* plants came from the union of seven chromosome gametes and the tetraploid from the union of 14 chromosome gametes. RENNER, however, is not impressed by this performance and holds to the possibility of the union of gametes with chromosomes seven and 21, or of gametes with chromosomes of intermediate numbers. He thus suggests a 21 chromosome gamete of which Oenothera research gives no evidence, or the union of gametes of intermediate numbers which would greatly decrease the probabilities of a zygote carrying four sets of *franciscana* chromosomes and consequently breeding true as did this tetraploid.

It can be proposed that a *franciscana* zygote from the union of *franciscana* gametes out of the triploid might have produced the tetraploid through chromosome doubling. Against this possibility is the record of my selfed *franciscana* line which since 1913 has been carried through 21 complete generations with a total of 6946 *franciscana* plants (diploid), three triploids, and four haploids, but no tetraploids. The triploids came from seeds from small capsules, the result of experiments on sparse pollination (DAVIS 1937, p. 103, table 2). This experiment indicated that pollen tubes of *franciscana* carrying diploid gamete nuclei grow more slowly than the normal and have little or no chance to effect fertilization under the usual conditions of excess pollination. Supporting this record is that of a selfed line derived from a haploid selfed which, carried through six generations, gave a total of 3922 *franciscana* plants and three haploids, but no triploids or tetraploids (DAVIS 1937, p. 105).

It seems probable that in Oenothera there are two methods by which tetraploids may arise: (1) By somatic doubling, possible from any diploid line and most likely when the origin is from an immediate cross; (2) through the union of diploid gametes, probable when the origin is from a triploid. It has been the writer's good fortune to study material believed to illustrate both types of origin, the first as recorded in this paper and the second in the account of the tetraploid *franciscana* (DAVIS 1933).

SUMMARY

The cross *franciscana*×*biennis* gives a progeny mostly of plants pale green and weak which die rapidly as seedlings and small rosettes. There is a small class of dark green seedlings that produce large strong rosettes and mature plants. Combined data from three large cultures gave 3672 pale green seedlings and rosettes and 44 dark green plants among which were three triploids and the amphidiploid.

The amphidiploid differed from the dark green diploids in having larger and thicker leaves, larger flowers, longer and stouter capsules, and pollen grains mostly four-lobed.

The dark green diploids have a chromosome configuration of a circle or chain of ten chromosomes and two pairs, or the ten chromosomes may be grouped in two or more smaller chains.

The amphidiploid at diakinesis presented a wide range of chromosome groupings. Complete pairing was rare. Usually there was a mixture of pairs, trisomes, and chains of four chromosomes.

Examples of regular distribution of the 28 chromosomes of the amphidiploid by the first meiotic division were not very common. Segregations of 13 and 15 were found with other irregularities leading to much abortive pollen. Fertile gametes were formed carrying 13 and 15 chromosomes, since the amphidiploid threw plants with 27 and 29 chromosomes.

The first generation from the amphidiploid presented an unexpectedly diverse progeny: (a) 11 plants with flowers larger than the amphidiploid (test plant No. 11, 28 chromosomes), (b) ten plants with medium-sized flowers, similar to the amphidiploid (test plant No. 34, 28 chromosomes), (c) eight dwarfs with medium-sized flowers, the sterile seeds with very little content (test plant No. 45, 29 chromosomes), and (d) two small-flowered plants (test plant No. 155, 27 chromosomes).

Second generations from the four selected plants gave: (a) from plant No. 11 (28 chromosomes, large flowers) a wide range of progeny in leaf form and flower size, a behavior similar to that of the parent amphidiploid; (b) from plant No. 34 (28 chromosomes, medium-sized flowers) a fairly uniform progeny, such as one would like to obtain from an amphidiploid; (c) from plant No. 45 (29 chromosomes, a dwarf) no germination from 90 seeds; (c) from plant No. 155 (27 chromosomes, small flowers) nine plants like the parent, 39 plants with medium-sized flowers, nine large-flowered plants.

Thus, this amphidiploid in the irregularities of its breeding showed itself to be far from a "constant species hybrid."

It is held that tetraploids in Oenothera may arise and in material studied by DAVIS have arisen both by somatic doubling (most likely when from diploids) and through the union of diploid gametes (more probable when the origin is through triploids).

Origin by way of somatic doubling seems more probable when a tetraploid appears directly from a cross as in this amphidiploid from *franciscana*×*biennis* and the five tetraploids from crosses obtained by RENNER (1933).

Origin through the union of diploid gametes, as held by STOMPS, is very much more likely when the tetraploid is from a triploid. From such an origin the tetraploid *franciscana* is believed to have arisen, the genetics and cytology of which was described by DAVIS (1933).

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