THE GENETICS AND CYTOLOGY OF A TETRAPLOID FROM OENOTHERA FRANCISCANA BARTLETT¹

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Tetraploid plants of *Oenothera* have appeared in a number of lines under investigation and from unrelated material. The first plant, *gigas* from *Lamarckiana*, was discovered by DE VRIES (1901) in 1895 and he records that *gigas* also arose from seed of *sublinearis* in 1898 and from the cross *lata* × *hirtella* in 1899 although the latter of these two plants did not mature. MACDOUGAL (1907) reports the appearance of one plant of *gigas* from his cultures of *Lamarckiana* and SCHOUTEN (1908) lists three plants from commercial seed of *Lamarckiana*. HUNGER (1913) from large cultures of *Lamarckiana* grown in Java records five plants of *gigas*. HERIBERT-NILSSON (1909, 1912) described a plant from Swedish *Lamarckiana* with characters chiefly those of *gigas*, and GATES (1915) determined its tetraploid chromosome count. GATES (1913) identified as *Lamarckiana gigas* a line from the botanic garden of Palermo and showed it to be tetraploid. A dwarf, *gigas nanella*, from the original line of *gigas* has been studied by DE VRIES (1901, 1915), and SCHOUTEN (1908) obtained a number of these plants. BOEDIJN (1920) established a dwarf tetraploid from *Lamarckiana* mut. *simplex* of DE VRIES. Making allowance for the fact that chromosome counts were not made on some of the plants early reported as *gigas* there are still substantial records of the appearance of this tetraploid from *Lamarckiana* through a number of different lines.

Tetraploids out of species of *Oenothera* other than *Lamarckiana* or its derivatives have been recorded from several sources, the most important of which are the following: BARTLETT (1915a, b) reported *gigas* from *Oenothera stenomeris* and *O. pratincola*. STOMPS (1916) reported tetraploids from the cross *Lamarckiana gigas* × *atrovirens*. A line of *grandiflora* gave tetraploid plants to DE VRIES (1918) and to BOEDIJN (1924). STOMPS (1925) obtained from a cross between *biennis* and *biennis cruciata*, a *biennis gigas*, and crosses of this tetraploid with *biennis cruciata* gave *biennis cruciata gigas*. Plants of *biennis cruciata gigas sulfurea* were shown by STOMPS in the exhibition garden of the Sixth International Congress of Genetics at Ithaca in 1932. There has also been recorded the appearance of tetraploids from crosses of *gigas* and *semigigas* with certain hybrids (BOEDIJN 1924). To this list may now be added a tetraploid by way of my line of *Oenothera franciscana* B, a species with all pairing chromosomes.

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With respect to the origin of the tetraploid *Oenotheras* there have been two principal and opposing views. GATES (1909a) held that "the double number of chromosomes in *O. gigas* originated soon after fertilization by the failure of a nucleus to complete its division after the chromosomes had divided, a condition comparable to the monasters of BOVERI," and this interpretation was supported by STRASBURGER (1910). This view was soon criticized by STOMPS (1912a, b) and by LUTZ (1912) and has not been favorably received by DE VRIES (1913) or generally by other workers on *Oenothera* cytology and genetics who believe with STOMPS that diploid gametes are developed and function. These diploid gametes may rarely meet to produce the tetraploid; more frequently they mate with normal gametes to give the well known triploid *Oenotheras* out of which tetraploids may come. The origin of my tetraploid from *franciscana* was by way of a triploid thus supporting the conclusions of STOMPS.

Triploid *Oenotheras* derived from *Lamarckiana* were first established through chromosome counts by STOMPS (1912a) and LUTZ (1912). STOMPS wrote of them under DARWIN'S term of *hero* plants and gave them the name *semigigas* and these names are found in many papers on *Oenothera*. Triploids may be expected to produce a much larger proportion of diploid gametes than do diploids and the chances of an origin of tetraploids by way of triploids are correspondingly greater than the possibilities of an origin from diploids direct. Recent cytological work on haploid *Oenotheras* by DAVIS and KULKARNI (1930), EMERSON (1929) and STOMPS (1930) has shown the manner by which meiosis may be eliminated from the history of pollen formation in *Oenothera*, and the early observation of GEERTS (1909, p. 52) of an embryo sac in *Lamarckiana* with 28 chromosomes appears to have been a case of similar suppression of meiosis in the ovule. Thus the failure of a heterotypic spindle to develop or to function with the consequent reconstitution of a nucleus which then passes through the homoeotypic division would give two diploid spores. Or, the suppression of the homoeotypic division following a normal heterotypic division, but after the lengthwise division of the chromosomes, would produce a diploid spore. Both of these irregularities give the same diploid chromosome set when they occur in homozygous material, but in heterozygous material spores from suppressed homoeotypic divisions would express such chromosome peculiarities as might result from segregation in the heterotypic division.

With respect to the possibility of the origin of triploids through dispermy we have the interesting report and figure of ISHIKAWA (1918) for *Oenothera nultans* $\times$ *O. pycnocarpa*, and the observations of NEMEC (1912) on *Gagea*. No evidence for dispermy was reported through the careful work of GEERTS (1909) and the studies of RENNER (1914) and others on the

embryo sac of *O. Lamarckiana* and other species. Neither is there known any genetical behavior of *Oenothera* triploids that would suggest an origin through dispermy. Since the investigations on dispermy in animal material have established irregularities of subsequent chromosome distribution and the failure of the embryo to attain maturity, usually with its early death, we may scarcely hope that dispermy will prove to be responsible for the vigorous triploids produced by plants. The rapidly increasing reports of the production of diploid gametes in plants through the suppression of the first or second divisions of meiosis offer the only evidence on which we are at present justified in drawing conclusions. GATES (1928) has, however, expressed the view that triploids have almost certainly arisen through dispermy.

There is apparently no *Oenothera* material known that supports the interesting views of WINGE (1917, 1932) on the origin of constant species-hybrids as tetraploids resulting from the doubling of the chromosome sets in the F_1 individual. The tetraploids of *Oenothera* which have come directly from diploids have behaved genetically, as far as reported, out of line with WINGE's hypothesis and it seems more probable that they have arisen through the union of diploid gametes. This matter will be considered in the Discussion.

The present study offers the first pedigree of a tetraploid *Oenothera* from a diploid by way of a triploid plant with cytological observations upon the plants directly concerned.

METHODS

Except for the F_1 generation from *franciscana* $\times$ *franciscana sulfurea nana*, grown at the JOHN INNES HORTICULTURAL INSTITUTION, all of the cultures were from seeds forced to germination over wet filter paper in Petri dishes following about 24 hours of alternate exhaust and pressure while soaking in vials of water. This treatment very much hastens germination apparently through the removal of air from the pores of the seed coats and the forcing of water against the contents of the seeds. Generally almost all of the viable seeds have germinated within 10 days and the remainder usually germinate during the next week. Seedlings are removed as they germinate to other Petri dishes and the residue in the first dish constitutes the mass of sterile seeds. When large enough to handle easily with the forceps the seedlings are planted in flats. There is little mortality among the seedlings, those that die being generally weaklings that are unable to free themselves without assistance from the seed coats. I greatly appreciate the kindness of Prof. BATESON who in 1925 gave space in the gardens at Merton for a set of cultures upon which I made observations and selfings.

It is fortunate that cytological material was collected from the triploid and tetraploid of this line so that the origin of the tetraploid is known from both the genetical and cytological side. I am indebted to Dr. ELLIOT WEIER for the material of the triploid (26.51-477) which was fixed in Strong Flemming and in Zenker's fluid, and for the material of the first tetraploid (27.36-18) fixed in Allen's modification of Bouin. The material of the second tetraploid (28.34-55), collected by Dr. KULKARNI, was fixed in a modification of Bouin somewhat stronger in chromic acid and urea and proved particularly good (Bouin's fluid 100 cc, 1 g chromic acid, 2 g urea).

Iron alum haematoxylin gave its usual dependable results but the best preparations were stained by a modification of the Gram method as recommended by NEWTON. The procedure for this material was as follows: (1) Stain for 20 minutes in a solution of crystal violet or gentian violet, 1 g in 100 cc water. (2) Rinse in water and then for 30 seconds move the slide about in a solution of 1 g iodine and 1 g potassium iodide in 100 cc 80 percent alcohol. (3) Carry slide very rapidly through alcohols to clove oil. (4) Remove clove oil with xylol. The differentiation of the stain takes place chiefly in clove oil. If it proceeds too rapidly hasten the dehydration and for clearing use a mixture of one-third clove oil and two-thirds xylol. If destaining is too slow alternate clove oil with absolute alcohol. Chromosomes should be violet and the cytoplasm almost colorless. The chief advantage of this stain over haematoxylin lies in the translucence of the chromatic material making it possible to focus into lower levels of crowded structures.

GENETICS OF THE TRIPLOID AND TETRAPLOID

For a number of years I have been gathering data on the inheritance of three characters of *Oenothera franciscana* Bartlett in contrast with their absence in a series of derivatives from an original cross between *franciscana* and *O. biennis* L. These characters are (1) petal color whether yellow or sulfur (a cream color), (2) stature whether tall or dwarf, and (3) color of bud cones whether with red or wholly green. In the course of this work there appeared a segregate which is sulfur in flower color and dwarf in stature, in contrast to *franciscana* which has yellow flowers and is tall; both forms have bud cones with red, the gene for which is linked with that for stature. The derivative will be called *franciscana sulfurea nana* and it differs from *franciscana* in the absence of one gene that produces yellow flowers and another gene that gives tall stature. I believe that each of these characters, color and stature, depends on the presence or absence of a single gene, but certain peculiar ratios of segregation from crosses have led to experiments not yet completed in an attempt to account for marked deficiencies of sulfur and some deficiencies of dwarfs among the segregates.

The genes for color and for stature are in different chromosomes since they segregate independently from all crosses in which they are brought together.

It is necessary to make this explanation because the triploid, established by cytological study, which produced the tetraploid was one of four similar "hero" plants in an F_2 generation from the cross *franciscana* $\times$ *franciscana sulfurea nana*. These hero plants (probably all triploids) have appeared in the progeny of a number of different crosses and also once in the selfed line of *franciscana* (culture 24.21a). The triploid then is not a very rare sport.

TEXT FIGURE 1.—*Oenothera franciscana gigas*, a tetraploid arising by way of a triploid selfed, the latter from the F_2 of the cross *franciscana* $\times$ *franciscana sulfurea nana*.

Both *franciscana* and *franciscana sulfurea nana* are homozygous with sets of 14 pairing chromosomes as shown for the first plant by studies of CLELAND (1922) and KULKARNI (1928), and for the second plant by a study of EMERSON (1928), observations which have several times been confirmed in our laboratory. These chromosomes segregate freely in meiosis. The pollen of both plants is 99 percent good and the seeds are about 90 percent viable.

With respect to the characters under consideration the genetic formula for *franciscana* may be expressed as *YYTT* and for *franciscana sulfurea nana* *yytt*; *Y* stands for yellow and *T* for tall, *y* for sulfur and *t* for dwarf.

The cross with which the line started was *franciscana* $\times$ *franciscana sulfurea* *nana*, and the F_1 generation, $YyTt$, was grown at the JOHN INNES HORTICULTURAL INSTITUTION in the summer of 1925. This culture matured 199 plants uniform and *franciscana*-like although a little taller and with somewhat larger leaves and flowers, the latter a slightly deeper shade of yellow perhaps due to thicker petals. The pollen of these F_1 plants was 99 percent good, and the seeds 95 percent viable.

The F_2 generation from seeds of a selfed F_1 plant, ($YyTt$), germination 95.3 percent, consisted of 643 seedlings (culture 26.51, table 1) of which 617 plants were brought to maturity. These were distributed as 498 yellow tall including the 4 hero plants (triploids), 98 yellow dwarf, 16 sulfur tall and 5 sulfur dwarf. The expected segregation, assuming complete fertility of gametes and zygotes, would be 1 sulfur to 3 yellow and 1 dwarf to 3 tall; the actual ratios were 1 sulfur to 28.4 yellow and 1 dwarf to 5 tall, a very marked deficiency of sulfur and a large deficiency of dwarf segregates.

The plant (26.51-477) selected from the four hero types for selfing and which cytological study showed to be a triploid (21 chromosomes) must have arisen from the union of a normal 7 chromosome gamete with a gamete carrying 14 chromosomes. Since the triploid was yellow tall and the tetraploid derived from it was yellow tall and both types threw no segregates for sulfur or dwarf it may be assumed that the 7 chromosome gamete was YT and the 14 chromosome gamete $YTYT$. The YT gamete, which is the normal gamete of *franciscana*, should constitute one fourth of the gametes developed by the F_1 hybrid $YyTt$. The $YTYT$ gamete could only have arisen through the suppression of the homoeotypic division of a YT nucleus segregated by the heterotypic division, the 7 chromosomes of which by their lengthwise division previous to interkinesis becoming 14 in number and the nucleus in consequence $YTYT$. Fortunately we know that such a suppression of the homoeotypic division through irregularities of spindle development may take place in *Oenothera* for it was observed in the haploid derived from *franciscana* (DAVIS and KULKARNI 1930, p. 73, figure 36).

The triploid plant (26.51-477) was easily distinguished in the field by its stature, taller than *franciscana*, its leaves larger, and by the much thicker bud cones. Only about 30 percent of the pollen in the triploid was well formed of which about half of the grains were 3-lobed and the other half 4-lobed with occasionally a 5-lobed example. The other grains were much shriveled and empty of material, and they also fell into two groups of 3-lobed and 4-lobed grains. Text figure 2 gives some examples of the pollen taken from this triploid plant (26.51-477) and for comparison there is included a pollen grain of *franciscana* (*f*) under the same magnification as the representatives of the triploid. It will be noted that the 4-lobed grains

are larger than those of *franciscana*; they probably carry chromosome counts above the 7 of *franciscana* but not the double set of 14. Pollen grains with 14 chromosomes from the tetraploid (2 sets of *franciscana*) as shown in text figure 3 are very much larger; they are formed very rarely and none chanced to be on the slide preserving the pollen from this triploid plant. Experience has shown that triploids in this material can be recognized with certainty by the peculiarities of the pollen as described above.

The triploid (26.51-477) selfed gave the small harvest of 513 seeds from 11 capsules, less than one-tenth the harvest of *franciscana*, and of these seeds only 116 proved viable, a germination of 22.6 percent. There was heavy mortality among the seedlings and young rosettes such that only

TEXT FIGURE 2.—Samples of pollen from the triploid plant (26.51-477), parent of the tetraploid (27.36-18). Of the 30 percent of good pollen about one-half of the grains were 3-lobed and the other half 4-lobed. A grain of *franciscana* (f) is shown for comparison. $\times 200$, reduced one-third.

89 plants lived (culture 27.36, table 1). These were distributed as 3 tetraploids, 42 *franciscana*, 17 dwarfs with narrow leaves and small flowers, and 27 dwarfs of a variety of form that failed to mature.

One of the three tetraploids (27.36-18), which will be called *Oenothera franciscana gigas*, was selected as parent of a tetraploid line and material was collected for the cytological study which established the count of 28 chromosomes. It must have arisen from the union of two 14 chromosome gametes each consisting of two sets of *franciscana* chromosomes. The tetraploid is then to be interpreted as carrying four sets of *franciscana* chromosomes and with respect to the factors for flower color and stature to have the formula $YTYTYTYT$. The 42 *franciscana* plants in the same culture (27.36, table 1) resulted from the union of gametes bearing the single *franciscana* set of 7 chromosomes. These conclusions are based on the findings of the cytological study of the triploid plant (26.51-477), parent of the

culture, in which segregation from the trivalents during meiosis gave occasionally a distribution of 14 but very much more frequently a distribution of 7 chromosomes. The mixed assemblage of dwarfs probably reflected irregularities of chromosome segregation characteristic of the triploid.

The tetraploid, text figure 1, is not quite so tall as *franciscana* but the leaves are much larger and thicker, the bud cones much broader, and the flowers larger. The capsules are shorter and thicker expressing a yield of seeds per capsule less than half that of *franciscana*. In general the tetraploid may be described as more robust than *franciscana* as may be seen by comparing text figure 1 with the published figure of *franciscana* (DAVIS and KULKARNI 1930, p. 57). The pollen is about 20 percent well formed and these grains have 4 or more lobes; very rarely a 3-lobed grain is produced. As shown in text figure 3 the good grains are generally larger than those of the triploid, text figure 2, and very much larger than the pollen grain of *franciscana* (f). The remainder of the pollen consists of shriveled empty grains of various sizes, mostly 4-lobed. As in the case of the triploid, experience has shown that the tetraploid may be recognized with certainty by the peculiarities of its pollen so that an examination of the pollen will separate tetraploids from triploids when other distinguishing characters are less clearly evident.

The first tetraploid plant (27.36-18) of the line produced 159 seeds from 5 capsules which gave a progeny of 59 seedlings (culture 28.34), a germination of 35.9 percent. There matured 41 tetraploids uniform and true to the type; 7 dwarfs failed to flower. The pollen of the plant (28.34-55) selfed for the next generation proved to be 10-20 percent good.

The second generation of the tetraploid line (culture 29.47), from 349 seeds out of two capsules, started with 227 seedlings, a germination of 65.3 percent. There matured 218 tetraploids, uniform; one variant, a broad-leaved dwarf, failed to flower. The plant (29.47-113) selfed for the next generation presented pollen 20 percent good.

The third generation of the tetraploid line (culture 30.41) from 210 seeds out of one capsule, began with 146 seedlings, a germination of 69.5 percent. There matured 129 tetraploids, uniform; 6 narrow-leaved dwarfs failed to flower; a single plant, *franciscana*-like, probably came from the parthenogenetic development of an egg. The plant (30.41-90) selfed to continue the line had pollen 20 percent good.

The fourth generation of the tetraploid line (culture 31.51) from 324 seeds out of one capsule started with 92 seedlings, a germination of 28.4 percent. There matured 54 tetraploids and one plant with narrower leaves. The plant (31.51-43) selfed for the next generation had pollen 10 percent good.

TABLE 1
Pedigree of the triploid and tetraploid.

CULTURE	PARENT	PERCENT OF GER- MINATION	SEEDLINGS	MATURE PLANTS	FAILED TO FLOWER	DIED	GOOD POLLEN IN SELFED PLANT	NUMBER OF SEEDS PER CAPSULE ON SELFED PLANT
F ₁ generation J.I.H.I. 1925	<i>franciscana</i> × <i>franciscana</i> <i>sulfurea nana</i>			199 <i>franciscana</i> -like	40 <i>franciscana</i> -like		99 percent	About 500
F ₂ generation (26.51)	F ₁ plant grown at J.I.H.I.	95.3	643	498 Yellow tall, including 4 triploids 98 Yellow dwarf. 16 Sulfur tall 5 Sulfur dwarf <u>617</u>	3 Dwarfs	23	Triploid (26.51-477) 30 percent	Triploid (26.51-477) About 50
From the triploid (27.36)	Triploid 26.51-477	22.6	116	3 Tetraploids 42 <i>franciscana</i> 17 Dwarfs, narrow-leaved, small-flowered <u>62</u>	27 Dwarfs	27	First tetraploid (27.36-18) 10 percent	First tetraploid (27.36-18) About 32
From first tetraploid (28.34)	Tetraploid 27.36-18	35.9	59	41 Tetraploids	7 Narrow-leaved dwarfs	11	Second tetraploid (28.34-55) 10-20 percent	Second tetraploid (28.34-55) About 175
From second tetraploid (29.47)	Tetraploid 28.34-55	65.3	227	218 Tetraploids	1 Broad-leaved dwarf	8	Third tetraploid (29.47-113) 20 percent	Third tetraploid (29.47-113) 20 percent
From third tetraploid (30.41)	Tetraploid (29.47-113)	69.5	146	129 Tetraploids	1 <i>franciscana</i> -like 6 Narrow-leaved dwarfs	10	Fourth tetraploid (30.41-90) 20 percent	Fourth tetraploid (30.41-90) About 300
From fourth tetraploid (31.51)	Tetraploid 30.41-90	28.4	92	54 Tetraploids 1 Narrower-leaved <u>55</u>		37	Fifth tetraploid (31.51-43) 10 percent	Fifth tetraploid (31.51-43) About 125

The chief points in this account of the history of the triploid and tetraploid are summarized in table 1. An interesting feature is the marked uniformity of the flowering populations from the tetraploids throughout the four generations. The few backward plants and dwarfs probably resulted from some unusual chromosome distributions, and the high percentages of shriveled pollen undoubtedly expressed in the main the irregularities of meiosis that break up the *franciscana* sets. The relatively small harvests of seed produced mean similar high percentages of sterility among the ovules. The true breeding of this tetraploid with respect to the plants that mature stands in marked contrast to that of *gigas* from *Lamarckiana* which always gives, when completely represented, a highly varied progeny. This contrast in behavior probably rests on the heterozygous constitution of the *gigas* derived from *Lamarckiana* in contrast to the more stable situation in the tetraploid from the homozygous *franciscana*.

CYTOLOGY OF THE TRIPLOID

There was not sufficient material of the triploid (26.51-477) to make profitable a study of the earlier prophase of meiosis in the microsporocyte. This account will then begin with the period of the second contraction which, as shown in plate 1, figure 1, presents a thick chromatic thread for the most part closely gathered but with some loops or arms extending outward from the central mass. The appearance suggests that synaptic associations have already taken place as becomes evident when the loops and other portions of the spirem separate as the nucleus passes out of second contraction. Segments of the chromatic thread are then clearly shown as chromosomes, in pairs and in groups of three (figures 2 and 3). Chains of three segments which are to become trivalents are numerous (figure 3), but no nucleus was observed in which the 21 chromosomes were in 7 chains of 3 each. Nevertheless, such must occur since 7 trisomes were found on a few spindles at metaphase of the heterotypic division (figure 5). Generally there is a mixture of pairs, groups of three, and solitary segments.

Further condensation of the segments gives the short chromosomes characteristic of the heterotypic division and these are assembled in association with a multipolar spindle (figure 4). The chromosomes are mostly in trivalents and bivalents which become very clear as the equatorial plate is established. This is as one would expect in a triploid with all pairing chromosomes. Occasionally a spindle carries 7 trivalents as shown in figure 5 where three of the trisomes from a lower focus are drawn at one side. Usually both bivalents and trivalents are present, as in figure 6, which gives 5 trisomes and 3 pairs; the members of one of the pairs must be a chance association of odd chromosomes that failed to mate with their homologues. Sometimes one or more chromosomes lie quite apart appar-

ently having failed to establish associations with others. The three chromosomes of a trivalent may be grouped in one of two ways as shown in figure 5; either (1) there may be a third chromosome attached to one member of a

ab

pair as *ab-ba* or (2) in a chain of three the middle chromosome is bent so that the two end chromosomes lie parallel although not in conjugation as *ab ab*. Either way it is evident that two chromosomes will pass to one

ba

pole and the third to the other pole. When 7 trisomes are present they rarely lie so that the segregation is 7 and 14 although such segregation must take place since nuclei were found at interkinesis with 7 split chromosomes (figure 8), and at the end of the homoeotypic mitosis with 14 chromosomes (figure 16). Usually the trivalents lie with some of the single chromosomes pointing to one pole and some to the other as illustrated in figure 5 where segregation would have given two groups of 9 and 12 chromosomes respectively. Assuming no irregularities of trisome formation and no lagging of chromosomes the mathematical probabilities of a segregation of 7 and 14 chromosomes from a set of 7 trisomes would be 1:128. Thus a very large percentage of the segregation is sure to be irregular even when a full set of trisomes is present.

Other examples of irregularities of segregation are shown in figures 7 and 9 where the distribution of the chromosomes is 10-11 and 8-13 respectively, and figures 10, 11, 12 and 13 give nuclei at interkinesis with counts of 9, 10, 11 and 12 chromosomes. Some of this irregularity results from the fact that very many heterotypic spindles present fewer trisomes than the maximum of 7, the other chromosomes being in pairs (figure 6), or loosely associated. On such spindles the chances of the segregation of sets of 7 and 14 chromosomes is much less than when the full number of trisomes is present.

Interkinesis (figures 8-13) presents the split chromosomes characteristic of *Oenothera* in preparation for the homoeotypic division. During prophase of this division the chromosomes become further condensed (figure 14), and at metaphase (figure 15), the daughter chromosomes are usually distributed in equal sets. Consequently a tetrad of microspores will generally have two nuclei with one chromosome number and two of another as determined by the heterotypic division, the sum of the two numbers being 21. Thus from the nucleus of figure 14 would have come two nuclei of a tetrad each with 9 chromosomes, and from the division of figure 15 two nuclei each with 12 chromosomes. Rarely are nuclei in tetrads found with 14 chromosomes (figure 16), but the union of gamete nuclei of this character produces the tetraploid.

Another factor leading to irregular distribution of chromosomes is the

lagging and consequent separation of occasional chromosomes from their proper groups. When this takes place on the heterotypic spindle there may be found during interkinesis 4 nuclei in a cell, two large and two small (figure 18), the latter containing the chromosomes that failed to reach the poles. Such small nuclei following the heterotypic division were not found to pass into the homoeotypic division. Similar small nuclei may be formed from chromosomes that lag during the homoeotypic division (figure 17). The final results of such failure of the normal distribution of chromosomes are sporocytes usually with 6 nuclei (figure 19), of which two are much smaller than the others. This gives the not uncommon condition of polypores such as have been reported for other triploids and for various hybrid material.

TEXT FIGURE 3.—Samples of pollen from the tetraploid plant (27.36-18), parent of the tetraploid line. The 20 percent of good pollen showed grains much larger than those of *franciscana* (f), and with 4 or more lobes. $\times 200$, reduced one-third.

The behavior of the chromosomes during meiosis in the triploid makes clear the breeding peculiarities of this plant. As shown by the records of culture 27.36 (table 1), the progeny from the triploid plant was 3 tetraploids, 42 *franciscana*, 17 dwarfs with narrow leaves and small flowers, and 27 dwarfs that failed to flower. The large and strong plants were then products of the union of 14 chromosome gametes to give tetraploids and the union of 7 chromosome gametes to give *franciscana*. We do not know the chromosome organization in the dwarfs but they probably came by way of gametes with chromosome counts between 7 and 14. The 70 percent of shriveled pollen presented by the triploid would seem to be amply covered by the irregularities of chromosome distribution during meiosis, the smallest shriveled grains being from cells with nuclei of low chromosome

count. Nuclei with large chromosome numbers might also produce shriveled grains if the chromosome assortments were of an unfavorable character.

In connection with the large number of dwarfs there must also be taken into consideration the high mortality of plants in the field (culture 27.36, table 1) and the low seed germination of 22.6 percent. These data indicate that many gametic combinations fail to give viable zygotes or produce weaklings. The large number of *franciscana* plants thrown by the triploid shows that the segregation of the 7 chromosomes constituting the *franciscana* set must take place with much greater frequency than the segregation of two sets of *franciscana* chromosomes necessary for the gametes that may produce the tetraploid. This is to be expected since the accidents of irregular distribution are more likely to lessen the frequency of the diploid sets of 14 chromosomes than of the haploid sets.

CYTOLOGY OF THE TETRAPLOID

The cytology of the tetraploid has been studied from two plants (27.36-18 and 28.34-55) of the first and second generation. Stages from the first plant are given in figures 20-29 and from the second plant in figures 30-34. The account of meiosis will make use of both sets of figures for there was no difference in the behavior of the two plants. The equatorial plate of a somatic mitosis from a developing petal with its 28 chromosomes is shown in figure 20.

The chromosomes emerge from second contraction in short chains as illustrated in figure 21 where about 22 chromosomes are evident, the remaining 6 probably being hidden in the central chromatic mass. The chromosomes then become more evenly distributed in a stage corresponding to diakinesis, but they are still in short chains or occasional pairs as shown in figure 22 where 23 chromosomes are now quite free and only 5 remain in the central group. No examples were found of complete pairing during diakinesis which must take place rarely if at all. An early spindle of the heterotypic division is presented in figure 23 where the chromosomes appear some in pairs and some in short chains. There seemed to be no marked tendency to form tetravalents although the tetraploid must contain 4 sets of homologous chromosomes.

Metaphase of the heterotypic division is shown in figure 24 with most of the chromosomes regularly paired but with trivalents and unpaired chromosomes also present. Figure 30 from the second tetraploid is particularly interesting because all of the 28 chromosomes could be drawn from different levels; there appear to be 7 pairs, a chain of 5, a chain of 4, a chain of 3, and 2 chromosomes probably not associated. The appearance of groups of 4 and 5 chromosomes probably means that short chains have

not yet broken apart as pairs of chromosomes. The large number of chromosomes apparently makes it difficult for all to find synaptic mates and consequently odd chromosomes are frequently present. These conditions lead to irregularities of meiosis and must be responsible for much of the bad pollen found in the tetraploids.

No heterotypic metaphases were observed with all of the chromosomes paired but such pairing and subsequent disjunction or at least a regular segregation must take place since counts of 14 chromosomes were found in nuclei at interkinesis (figure 25). An early telophase of the heterotypic division is shown in figure 31 with 14 chromosomes in each daughter nucleus. Prophases of the homoeotypic division with 14 split chromosomes are illustrated in figures 26 and 27, and in figure 32 a metaphase is shown with 14 split chromosomes on each of the two spindles which lay at right angles to one another. A telophase of the homoeotypic division with 14 chromosomes is given in figure 33. These examples show conditions that would lead to the production of the very small amount of pollen with 14 chromosomes capable of reproducing the tetraploid line.

There is much distribution of chromosomes by the heterotypic division in counts above and below the number 14 but judging from the highly true breeding of the tetraploid such chromosome groups apparently rarely produce fertile pollen. The failure of chromosomes to find their synaptic mates is responsible as in the triploid for a large amount of lagging on the heterotypic spindles and this results in irregularities of segregation. Lagging also occurs in the homoeotypic divisions (figure 28), similar to that which has been described for the triploid, and small nuclei may be developed (figure 29), with the consequent formation of polyspores. There seems to be enough of these irregularities to account for the large proportions of shriveled pollen grains.

It is rather surprising that the tetraploid should present a smaller percentage of good pollen than the triploid. Perhaps the explanation lies in the larger number of chromosomes concerned which make it less easy for a chromosome to meet its appropriate mate. The second, third and fourth tetraploids of the line (table 1) apparently had better pollen than the first and also produced more seeds per capsule, but the fifth tetraploid was about as highly sterile as the first so there is no evidence of improved fertility in the line.

There is shown in figure 34 a sporocyte which apparently contains a restitution nucleus following the suppression of the heterotypic division, in which case the nucleus should contain 28 chromosomes. The chromatin is much distributed and there is no indication of the approach of the homoeotypic division. The example, however, suggests that irregularity characteristic of the haploid from *franciscana* (DAVIS and KULKARNI

1930) by which two pollen grains may be formed in a sporocyte following the suppression of the heterotypic division.

The stability of the tetraploid line is remarkable. The few variants that appeared (table 1) were almost all dwarfs that failed to flower. The parent *franciscana* so regularly thrown by the triploid has not been represented in the cultures of the tetraploid which means that it is difficult if not impossible to segregate a set of the 7 *franciscana* chromosomes from the 28 chromosomes or four sets in the tetraploid. The homologous chromosomes of the tetraploid endeavor to associate in pairs and there appears to be no opportunity for a single set of 7 to detach itself from so large a group. This will, however, happen in the triploid whenever a full set of trisomes are so placed on the heterotypic spindle that the third chromosomes of the groups all face one pole.

Variations of chromosome segregation in the tetraploid present themselves in numbers greater or less than 14 rather than between 7 and 14 as in the triploid. Such irregularities of chromosome distribution, expressed through these higher numbers, might be expected to result in greater disturbances of cell physiology with the consequent production of a higher proportion of shriveled pollen in the tetraploid than in the triploid, and this is markedly the case.

DISCUSSION

We have noted that the first triploids observed to arise directly from an *Oenothera* species were those from *Lamarckiana* established by chromosome counts of STOMPS (1912a) and LUTZ (1912). LUTZ also obtained triploids from *lata* and from *lata* $\times$ *Lamarckiana*, and STOMPS (1912b) reported a triploid from the cross *biennis cruciata* $\times$ *biennis*. These were important observations since they led to a series of studies which have established the fact that species of *Oenothera* not infrequently produce triploid sports in contrast to an early contrary opinion of GATES (1913). Later studies of which those on *Morus*, *Datura*, *Hyacinthus*, *Tulipa*, *Solanum*, *Zea*, *Crepis*, and *Petunia* are examples have shown that triploids are not rare in various groups of plants and support the conclusions on their importance as a stage in the development of higher polyploids.

The first study of the distribution at meiosis of the 21 chromosomes of a triploid *Oenothera* was that of GATES (1909b) on the hybrid *lata* $\times$ *gigas*. He described a segregation of chromosomes during pollen formation usually of 10 and 11, but occasionally of 9 and 12. In a later paper on the cross *rubricalyx* $\times$ *gigas* GATES (1923) again records the usual segregation as 10 and 11 with, however, irregularities that result from the lagging of chromosomes. In none of these studies on triploids did GATES describe or clearly figure the pairing of chromosomes at the time of the heterotypic division.

In this respect a paper of GEERTS (1911) on the cross *lata* × *gigas* stands in contrast to the observations of GATES. GEERTS reported a pairing between two set of 7 chromosomes with 7 chromosomes unpaired both in vegetative cells and at the heterotypic division. The behavior, therefore, was described by GEERTS as of the *Drosera* type, but unfortunately the study was not developed in detail. VAN OVEREM (1922) for *semigigas* also reported as usual a grouping of chromosomes in 7 pairs with the remaining 7 in a scattered distribution. There is general agreement of these investigators and also of later workers with the conclusions of GATES that the more common segregation of chromosomes is that of 10 and 11, but genetical studies indicate that the small percentages of fertile gametes produced are chiefly in a group of lower chromosome numbers, 7 or 8, or in the diploid number of 14 chromosomes.

A triploid from *Oenothera pycnocarpa* stated by CATCHESIDE (1930) to segregate its chromosomes, usually 10 and 11 or more rarely 9 and 12, was also described as presenting at diakinesis a closed chain of 21 chromosomes. Such an arrangement, if true, would be fundamentally antagonistic to the theory of segmental interchange between non-homologous chromosomes as applied to *Oenothera* cytology and genetics. This account of a closed chain of 21 chromosomes was promptly challenged by DARLINGTON (1930b) and by CAPINPIN (1930), and DARLINGTON after an examination of CATCHESIDE's slides interpreted them as showing various associations of chromosomes in pairs, in trisomes, and in other arrangements including single elements. CATCHESIDE (1931), accepting the interpretations of DARLINGTON, has abandoned his position.

The first descriptions of trivalent chromosomes in *Oenothera* material were those of HAKANSSON (1926, 1930, 1931) for the triploid plant *Lamarckiana excelsa* and for certain trisomic derivatives from *Lamarckiana*. CAPINPIN (1930) is to report in detail on a number of triploids from *Lamarckiana* and other species such as *franciscana*, *ammophila*, *biennis* et cetera, none of the triploids being derived from crosses involving either triploid or tetraploid forms. CATCHESIDE (1931) in the revision of his studies on the triploid from *O. pycnocarpa* gives good figures of chromosome associations, many of them trivalents, and an excellent discussion of their significance as possible products from a structural hybrid. The present study describes trivalents as frequent in a triploid sport from the homozygous *franciscana*, a species with all pairing chromosomes.

Thus *Oenothera* in presenting trivalents at meiosis in triploids agrees with the studies on *Canna* (BELLING 1921), *Datura* (BELLING and BLAKESLEE 1922), *Hyacinthus* (BELLING 1925a, DARLINGTON 1929b), *Hemerocallis* (BELLING 1925b), *Campanula* (GAIRDNER 1926), *Solanum* (LESLEY 1926, JÖRGENSEN 1928), *Zea* (RANDOLPH and MCCLINTOCK 1926, Mc-

CLINTOCK 1929), *Prunus* (OKABE 1928, DARLINGTON 1928), *Tulipa* (NEWTON and DARLINGTON 1929), *Narcissus* (NAGAO 1929), *Crepis* (NAVASHIN 1929), *Lycoris* (INARIYAMA 1931), *Petunia* (DERMEN 1931, STEERE 1932). These accounts of meiosis in triploids report no conspicuous regularity in the association of the chromosomes to form trivalents. There are few figures that show complete sets of trivalents such as are presented by BELLING for *Canna* and *Hyacinthus*, by MCCLINTOCK for *Zea*, by STEERE for *Petunia*, and illustrated for *Oenothera* in figure 5 of the present paper. The accounts generally describe and the figures present trivalents together with bivalents and univalents. Naturally the results of meiosis are most varied with the chromosomes segregated through a wide range of numbers. The lagging of chromosomes is common so that the full number frequently is not distributed to the two daughter nuclei following the heterotypic division and the lagging chromosomes may organize independent micronuclei. In consequence of these irregularities of chromosome distribution very large amounts of sterile pollen are formed.

With respect to the associations of chromosomes at meiosis in tetraploids they are bound to differ with the make up of the material whether auto- or allotetraploid. Allotetraploids with sets of dissimilar chromosomes are not likely to form quadrivalents at meiosis but autotetraploids with their four sets of homologous chromosomes may be expected to show tetrasomes. Tetravalents have been described and figured for *Datura* (BELLING and BLAKESLEE 1924), *Aucuba* (MEURMAN 1929), *Solanum* (JÖRGENSEN 1928, LESLEY and LESLEY 1930, LINDSTROM and KOOS 1931), *Prunus* (DARLINGTON 1928), *Hyacinthus* (DARLINGTON 1929b), *Pyrus* (DARLINGTON and MOFFETT 1930), *Dahlia* (LAWRENCE 1929, 1931), *Primula sinensis* (DARLINGTON 1931), *Rosa relict*a (ERLANSON 1931). The proportions of tetravalents present are widely various. *Datura* may present complete sets but in most material bivalents are found together with the quadrivalents which may be few in number.

The tetraploid from *Oenothera franciscana* failed to show more than occasional groupings of chromosomes in fours and these were in open chains at diakinesis (figures 22 and 23) and on the heterotypic spindle (figure 30). Such open chains readily break up into pairs which is the usual association of chromosomes in the metaphase of the heterotypic division. The failure of this autotetraploid to form marked tetrasomes and the predominance of pairs does not support the views on the importance of tetravalents, but rather strengthens the reports on other tetraploids where much irregularity of chromosome association is described. In this tetraploid *Oenothera* it is very common to find chromosomes which, having failed to meet with a mate, either associate with a pair to form a trisome (figure 24) or lie apart. The large numbers of chromosomes involved make probable a high per-

centage of such failures in synapsis with consequent irregularities of segregation.

Tetraploids from ring-forming species of *Oenothera*, which are structural hybrids, present conditions out of which disomes, tetrasomes, or chains might appear. Assuming *gigas* out of *Lamarckiana* (with its circle of 12 and one pair) to have the formula

ab	cd	ef	gh	ij	kl	mn
ab	cd	ef	gh	ij	kl	mn
bc	de	fg	hi	jk	la	mn
bc	de	fg	hi	jk	la	mn

the simplest arrangement of the chromosomes in synapsis would seem to be pairs such as ab ab, bc bc, cd cd, et cetera since both ends of each member of the pairs have homologues; the 4 chromosomes mn might be either as two pairs or as a tetrasome. However, chains might arise as for example cb ba ab bc which could be open or closed, short or long, possibly even up to the full number of the 24 chromosomes with interchanged segments. The only figures of *gigas* published are those of GATES (1911) and DAVIS (1911) and these indicate short chains and pairs during diakinesis and at metaphase of the heterotypic division. A re-examination of my slides supports these conclusions and the fact that *gigas* selfed gives a complex and diverse progeny indicates that the variants resulted from the numerous possibilities of irregular chromosome distribution.

In this account of the origin of a tetraploid from *Oenothera franciscana* by way of a triploid it is clear that the event must have taken place through the union of diploid gametes. There are only a few other accounts of the origin of tetraploid *Oenotheras* from hero plants, presumably triploids. DE VRIES (1923) gives the first record of a *gigas* individual by way of a plant of *semigigas* from a cross between *O. Lamarckiana* mut. *simplex* and *O. biennis* (Chicago). BOEDIJN (1924) reports *gigas* from *semigigas* plants out of the cross *lata* $\times$ *Lamarckiana*.

In contrast to the above are a number of accounts of tetraploid *Oenotheras* derived directly from diploid plants. The original *gigas* from *Lamarckiana* apparently had such an origin in the cultures of DE VRIES and other reports of *gigas* from *Lamarckiana* and from certain of its mutants give no suggestion of intermediary triploid plants before the appearance of the tetraploid. BARTLETT (1915a, b) obtained tetraploids directly from *O. stenomerus* and *O. pratincola*. DE VRIES (1918) recorded a tetraploid plant out of a line of *O. grandiflora*, also reporting *semigigas* from the same line, and *gigas* appeared in certain crosses; BOEDIJN (1924) added to this study. A tetraploid from *O. simplex nanella* of DE VRIES was established by BOEDIJN (1920). Other tetraploids have been reported by BOEDIJN as arising directly from crosses between hybrids and *gigas*. STOMPS (1925) ob-

tained a *biennis gigas* from a cross between *biennis* and *biennis cruciata*, and a *biennis cruciata gigas* from crosses of this tetraploid with the diploid *biennis cruciata*.

In view of the records of tetraploid *Oenotheras* out of diploid parentage it is not surprising that the hypothesis has been held by GATES and others of an origin after fertilization by a doubling of the chromosome number. This hypothesis of chromosome doubling is now best known through the extended discussion of WINGE (1917, 1932) who holds that a lack of harmony between the two sets of chromosomes in the F_1 of a wide cross might be compensated for by an "indirect chromosome binding" of daughter chromosomes following a division somewhere in the germ path, possibly in the zygote. We can conceive such a doubling to be brought about (1) by the failure of a mitosis to separate its daughter chromosomes with the immediate organization of a restitution nucleus, (2) by the fusion of two daughter nuclei, or (3) by the union of two spindles of sister nuclei lying side by side to form one spindle (WINKLER 1916) as assumed by the "endo-duplication" hypothesis of JÖRGENSEN (1928). These possibilities have their chief interest in relation to the occasional appearance of stable tetraploids, which are in effect new species, in the F_1 generations from crosses generally wide in character.

WINGE (1932) has assembled a number of cases of such stable tetraploids which apparently have arisen through the doubling of chromosome sets in the germ path of the hybrid. Good examples of this behavior are the cases of the tetraploid *Primula Kewensis* (NEWTON and PELLEW 1929), *Rosa Wilsoni* (BLACKBURN and HARRISON 1924), *Nicotiana digluta* (CLAUSEN and GOODSPEED 1925), a tetraploid from the cross *Fragaria bracteata* $\times$ *F. Helleri* (ICHIJIMA 1926), *Saxifraga Potternensis* (MARSDEN-JONES and TURRILL 1930), *Brassica napocampestris* (FRANDSEN and WINGE 1932). Outstanding features of these "constant hybrids" are their fertility and the absence of segregation which result from the harmonious relations established between the sets of chromosomes now doubled.

Support for WINGE's hypothesis of the origin of constant hybrids through the doubling of chromosome sets in the germ path is offered in the remarkable experiments on the decapitation of stems in *Solanum*. Tetraploid shoots together with diploid arise from meristematic tissue in association with the callus over the wound (WINKLER 1916, JÖRGENSEN 1928, SANSOME 1930, LINDSTROM and KOOS 1931, and LINDSTROM 1932). LINDSTROM and KOOS present evidence of nuclear fusions in binucleate cells in the callus of decapitated haploids that may produce diploid shoots. An extensive literature reports the formation of nuclei with higher chromosome numbers in root tips and other tissues following experimental interference with mitosis through effects of chemicals, by wounding and by high

temperatures. Binucleate cells have been described and figured in a wide range of plant material. Tetraploid branches have appeared rather frequently in the extensive cultures of *Datura Stramonim* (BLAKESLEE and BELLING 1924). SCHWEMMLE (1928) has reported fertile tetraploid shoots on an F_1 plant of *Oenothera Berteriana* $\times$ *O. odorata*.

There are, however, serious objections to the acceptance of WINGE's hypothesis of "indirect chromosome binding" or somatic doubling as an explanation of the origin of those tetraploid *Oenotheras* that have arisen directly from diploids. By such origin the tetraploids should breed true and may be expected to be highly fertile since their nuclei carry two sets of homologous chromosomes brought together by the suppression of mitoses or through the fusion of sister nuclei either at rest or during simultaneous mitoses. Now such information as we have on the tetraploid *Oenotheras* out of diploids indicate that many of them were far from that condition of genetic stability to be expected on the hypothesis of the duplication of the chromosome sets. The *gigas* from *Lamarckiana* is well known to breed very far from true, and widely varied progenies were obtained from the Swedish *gigantea* of HERIBERT-NILSSON (1912) and the *gigas* from Palermo studied by GATES (1915). The *gigas* out of *stenomeres* (BARTLETT 1915a) produced 54 *gigas* and 9 variants in a culture of 102 plants; the remaining plants died or failed to flower indicating a high proportion of atypical forms. The *gigas* from *pratincola* (BARTLETT 1915b) gave 16 dwarfs in a culture of 25; the other 9 plants died. The *gigas* from *grandiflora* (DE VRIES 1918, BOEDIJN 1924) repeated the performance of the line in giving the forms *lorea* and *ochracea*. The *biennis gigas* (STOMPS 1925) threw dwarfs.

Thus we are forced to consider the probabilities of an origin of these tetraploid *Oenotheras* out of diploids through the union of diploid gametes which might result from the suppression of either the first or the second of the divisions of meiosis. The suppression of the first division permits of no segregation and the two diploid pollen grains formed give gametes which should carry the exact chromosome constitution of the diploid parent. Union of such gametes would give doubled sets of chromosomes and should a regular pairing result the tetraploid might breed true and the results would not be different from somatic doubling.

The suppression of the second division of meiosis also gives two diploid pollen grains but these will be genetically different when derived from heterozygous stock since the first division will have effected a segregation of chromosomes. There is less information on the suppression of the homoeotypic division than on the heterotypic which has been reported for material in many groups of plants and is not an uncommon phenomenon. Evidence of the suppression of the homoeotypic division is given for Pru-

nus (DARLINGTON 1930a), in hybrids of Papaver (YASUI 1931, figure 26), for Triticum hybrids (THOMPSON 1931), and for Oenothera (DAVIS and KULKARNI 1930, figures 36 and 37). HAKANSSON (1929) reports the fusion of homoeotypic spindles in *Salix viminalis* $\times$ *S. caprea*. There is probably much more of this suppression of the second division of meiosis than this scant record would suggest; studies have not been particularly directed to it.

The species of Oenothera which have given tetraploids out of diploids are probably all chain-forming types in some degree and therefore structural hybrids. Tetraploids derived from them by way of diploid gametes formed through the suppression of the second division of meiosis could hardly fail to give indications through their breeding behavior of heterozygous conditions in the parent diploids. While the union of diploid gametes formed by the suppression of the first division of meiosis may be expected to give doubled sets of chromosomes we must grant the possibility of some variation among the gametes through rearrangements of chromosome segments in the prophases of meiosis and the consequent possibility of varied progeny. The evidence in the breeding records that have come to us indicates heterozygous conditions in the tetraploids under consideration. This is the argument for the conclusion that these tetraploid Oenotheras out of diploids have arisen through the union of diploid gametes and not by a process of somatic doubling.

SUMMARY

1. An autotetraploid of *Oenothera franciscana* Bartlett has arisen by way of a triploid in the F_2 of the cross *franciscana* (*YTYT*) $\times$ *franciscana sulfurea nana* (*yytl*).

2. The F_1 of this cross was uniform, all plants (*YyTt*) were *franciscana*-like, yellow dominant over sulfur, tall over dwarf.

3. The F_2 generation (table 1) gave the expected segregation of yellow tall, yellow dwarf, sulfur tall and sulfur dwarf through combinations of the gametes *YT*, *Yt*, *yT*, *yt*. Among the yellow tall were 4 "hero" plants (probably all triploids), one of which (26.51-477) was established to be a triploid, 21 chromosomes.

4. Since the tetraploid has failed to segregate either sulfur or dwarf this triploid must have arisen from the union of a 7 chromosome *franciscana* gamete (*YT*) with a 14 chromosome gamete (*YTYT*), the latter resulting from the suppression of the homoeotypic division of a *YT* nucleus and consequently including two sets of *franciscana* chromosomes.

5. The triploid (*YTYTYT*) selfed gave a progeny (table 1) of 42 *franciscana*, 44 various dwarfs, and 3 thick-leaved yellow tall plants (probably all tetraploids), one of which (27.36-18) was established to be tetrap-

loid, 28 chromosomes. The tetraploid must have come through the union of two 14 chromosome gametes and consequently carried four sets of *franciscana* chromosomes. The tetraploid has bred remarkably true through four generations (table 1).

6. The pollen of *franciscana* is 99 percent good; the capsules set about 500 seeds, 90 percent viable. The pollen of *franciscana sulfurea nana* is equally good, capsules set about 250 seeds, equally viable. The pollen of the F₁ plants was also 99 percent good, the capsules set about 500 seeds, and the germination was equally high.

7. The pollen of the triploid was about 30 percent good and about equally divided between 3- and 4-lobed grains (text figure 2); the triploid produced about 50 seeds to the capsule of which 22 percent proved to be viable.

8. The pollen of the tetraploid is about 20 percent well formed; the 4- or more lobed grains are larger than those of the triploid and very much larger than those of *franciscana* (text figure 3); the capsules set about 200 seeds moderately viable.

9. The 21 chromosomes of the triploid emerge from second contraction in pairs and in trisomes with occasional free chromosomes. If 7 trisomes are present they may lie so that 7 chromosomes pass to one pole and 14 to the other. Such segregation is not common but it must occur since nuclei were found in interkinesis and in the homoeotypic division with 7 and 14 chromosomes respectively (figures 8 and 16). Gametes with 7 chromosomes should on union give *franciscana* in agreement with the genetical findings. Union of 14 chromosome gametes should produce the tetraploid.

10. Most trisomes lie on the heterotypic spindle of the triploid with the third chromosome pointing sometimes to one pole and sometimes to the other giving various numerical distributions of the chromosomes (figure 5). There are also the pairs and odd chromosomes to effect irregularities of segregation together with lagging chromosomes that fail to reach the poles. Interkinesis in the triploid presents the split chromosomes characteristic of *Oenothera* but the lagging of chromosomes in the homoeotypic division frequently disturbs further the process of meiosis.

11. These irregularities of meiosis in the triploid would seem sufficient to account for the pollen sterility of about 70 percent and for the low seed germination of about 20 percent together with large numbers of dwarfs and the high mortality among the plants in the field since it is evident that many gametic combinations fail to give viable zygotes or produce weaklings.

12. The 28 chromosomes of the tetraploid are found mostly paired at metaphase of the heterotypic division but trisomes and odd chromosomes may be present and tetrasomes are occasionally represented by short

chains (figure 30). A regular segregation of the chromosomes does take place to a limited degree since counts of 14 chromosomes were found at various stages following the heterotypic division and from such nuclei must come the small percentage of fertile pollen.

13. There is much distribution of chromosomes through meiosis in the tetraploid in counts below the number 14 as a result of lagging and through failure of many chromosomes to find a synaptic mate. These irregularities are responsible for the large amount of bad pollen in the tetraploid which is greater than in the triploid, possibly because the larger number of chromosomes present makes it less easy for them appropriately to pair.

14. A sporocyte of the tetraploid was found (figure 34) which apparently contained a restitution nucleus following the suppression of the heterotypic division.

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EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 (num. aper. 1.30) in combination with the compensating oculars K20 or K10. In reproduction the figures are reduced one-third from the magnification of 1600 diameters except for figures 18, 19 and 34 where the reduction is from 800 diameters.

PLATE 1

FIGURE 1.—Triploid. Second contraction, synaptic associations indicated.

FIGURE 2.—Triploid. Chromosomes shortly after second contraction, a short chain of four is shown together with pairs.

FIGURE 3.—Triploid. The period corresponding to diakinesis. Trivalents are present together with bivalents.

FIGURE 4.—Triploid. Multipolar spindle of the heterotypic division showing trivalents and bivalents.

FIGURE 5.—Triploid. Metaphase of the heterotypic division with 7 trivalents. As arranged 12 chromosomes would pass to one pole and 9 chromosomes to the other.

FIGURE 6.—Triploid. Metaphase of the heterotypic division, 5 trivalents and 3 bivalents.

FIGURE 7.—Triploid. Anaphase of the heterotypic division, 11 chromosomes at one pole and 10 at the other.

FIGURE 8.—Triploid. Interkinesis, 7 split chromosomes.

FIGURE 9.—Triploid. Interkinesis, 8 split chromosomes in one nucleus and 13 in the other.

FIGURE 10.—Triploid. Interkinesis, 9 split chromosomes.

FIGURE 11.—Triploid. Interkinesis, 10 split chromosomes.

FIGURE 12.—Triploid. Interkinesis, 11 split chromosomes.

FIGURE 13.—Triploid. Interkinesis, 12 split chromosomes.

PLATE 2

FIGURE 14.—Triploid. Early spindle of homoeotypic division, 9 split chromosomes.

FIGURE 15.—Triploid. Metaphase of homoeotypic division, 12 split chromosomes.

FIGURE 16.—Triploid. Telophase of homoeotypic division, 14 chromosomes. The union of two gamete nuclei of this character would produce the tetraploid.

FIGURE 17.—Triploid. Heterotypic anaphase with lagging chromosomes which would probably have organized two micronuclei.

FIGURE 18.—Triploid. Interkinesis with two meganuclei and two micronuclei, the latter derived from lagging chromosomes such as are shown in figure 17.

FIGURE 19.—Triploid. Polyspores, the result of lagging chromosomes that form micronuclei.

FIGURE 20.—First tetraploid. Somatic mitosis from developing petal, 28 chromosomes.

FIGURE 21.—First tetraploid. Chromosomes emerging from second contraction in short chains, about 22 evident.

FIGURE 22.—First tetraploid. Early diakinesis, chromosomes in short chains, 23 clearly defined, probably 5 in the central group.

FIGURE 23.—First tetraploid. Early spindle heterotypic division, chromosomes in pairs and in short chains.

FIGURE 24.—First tetraploid. Metaphase of heterotypic division, chromosomes mostly bivalents, some trivalents and univalents.

FIGURE 25.—First tetraploid. Interkinesis, 14 split chromosomes.

PLATE 3

FIGURE 26.—First tetraploid. Prophase homoeotypic division, 14 split chromosomes.

FIGURE 27.—First tetraploid. Multipolar spindle homoeotypic division, 14 split chromosomes.

FIGURE 28.—First tetraploid. Homoeotypic telophase, lagging chromosomes between the daughter nuclei.

FIGURE 29.—First tetraploid. Mega- and micronuclei following the homoeotypic division, the former with 12 chromosomes, the latter probably formed from 2 lagging chromosomes.

FIGURE 30.—Second tetraploid. Metaphase heterotypic division, the 28 chromosomes drawn at different foci; there appear to be 7 pairs, a chain of 5, a chain of 4, a chain of 3, and 2 chromosomes probably not associated.

FIGURE 31.—Second tetraploid. Early telophase heterotypic division, 14 chromosomes in each daughter nucleus, a zone of starch grains between the daughter nuclei.

FIGURE 32.—Second tetraploid. Early homoeotypic division the two spindles at right angles to one another, 14 split chromosomes on each spindle. Such a sporocyte should produce a tetrad of fertile pollen grains of the tetraploid genotype.

FIGURE 33.—Second tetraploid. Early homoeotypic anaphase, 14 chromosomes. This should become the nucleus of a fertile pollen grain of the tetraploid genotype.

FIGURE 34.—Second tetraploid. Apparently a sporocyte in which the heterotypic division has been suppressed giving a very large restitution nucleus.