# THE CYTOLOGY AND GENETICS OF A HAPLOID SPORT FROM *OENOTHERA FRANCISCANA*[1]

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The first haploid spermatophyte to be recorded was from *Datura stramonium* (BLAKESLEE and others 1922), and later studies have shown them to be not infrequent sports from this species apparently appearing without marked relation to the temperature of the season. They have also been obtained by the application of pollen from *Datura ferox* to the stigma of *D. stramonium*. BELLING and BLAKESLEE (1923, 1927) presented a cytological study of these plants. Shortly after, CLAUSEN and MANN (1924) described two haploid plants from the $F_1$ of the cross *Nicotiana tabacum* $\times$*N. sylvestris*, and later CHIPMAN and GOODSPEED (1927) gave an account of the haploid from *Nicotiana tabacum* var. *purpurea*. RUTTLE (1928) has reported three additional haploids from *tabacum* var. *purpurea* and two new plants from *tabacum* var. *Cuba*. GAINES and AASE (1927) in attempts to pollinate a hybrid of Triticum from Aegilops obtained a haploid wheat plant and described its cytology. LESLEY and FROST (1928) found a dwarf plant of Mattiola having the haploid set of normal chromosomes and in addition a chromosome fragment. HOLLINGSHEAD (1928) records two haploids of *Crepis capillaris* from the $F_1$ of the cross *capillaris*$\times$*tectorum*. JÖRGENSEN (1928) has obtained a number of haploids of *Solanum nigrum* and *S. nigrum gracile* through capsules stimulated to parthenocarpic development by pollinations from *S. luteum* and other forms that hybridize only rarely with *S. nigrum*. JÖRGENSEN gives an account of the cytology of these Solanum haploids in which the behavior at meiosis is very different from that in Triticum, Nicotiana, Datura and as we shall see in Oenothera. LINDSTROM (1929) described a haploid from tomato almost completely pollen sterile but producing a few seeds after pollinations from other varieties. Our paper adds to the list the first haploid Oenothera but it seems probable that other plants of this character will shortly be recognized in this genus.

The possibility of apomixis in Oenothera has been strongly indicated by the interesting results of HABERLANDT (1921, 1922, 1927) in connection

[1] Cytological Studies on Oenothera IV. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 303.

with his experiments on the stimulus to growth resulting from wounds. By pinching the ovaries, by pricking them with a needle, and by the castration of young flowers he obtained early stages of parthenogenetic embryos and adventitious embryos in *Oenothera Lamarckiana*, together with endosperm development. HABERLANDT holds that the exciting agents are hormones liberated as the result of the wounding of tissue or the death of cells. Similar developments were noted in ovaries of *Lamarckiana* and *muricata* at the end of the flowering season and are believed by him to result from products formed by the aging plants.

There have been other suggestions of possible apomixis in Oenothera. LUTZ (1909) reported two plants of *lata* (15 chromosomes) in the $F_1$ of the cross *lata*×*gigas*. GATES (1909, 1924) performed experiments on *Lamarckiana-lata*, *biennis-lata* and *gigas* but obtained no seed. The brief statement of HAIG-THOMAS (1913) of parthenogenesis in *Oneothera biennis* following the castration of young flower buds can scarcely be accepted as a demonstration. It may happen that parthenogenesis in Oenothera will result, as JÖRGENSEN (1928) found in Solanum, through pollinations from types that will induce parthenocarpy or through a very limited amount of self pollination sufficient to start the development of fruit and with this growth give physiological conditions favorable to forms of apomixis. The well known reactions of many plants as expressed in parthenocarpy show a close physiological relation between the development of fruit and stimuli from pollen tube growth. The maturation of seed in many forms is apparently only possible as it proceeds hand in hand with the development of the fruit.

## GENETICS OF THE HAPLOID

*Oenothera franciscana* Bartlett, text figure 1, is one of the few apparently homozygous forms in this genus of numerous impure or heterozygous species. It is a large vigorous plant with pollen almost wholly perfect, text figure 3A, and with high seed fertility (about 90 percent). As would be expected its chromosomes are characteristically paired at diakinesis and during the first meiotic division. In certain material (CLELAND 1922) a chain of four chromosomes has been found persisting even to metaphase of the heterotypic mitosis but in other material (Kulkarni 1929) this chain breaks into two pairs at diakinesis showing the association to be loose in character. For eight years cultures with a total of 1499 plants gave no exceptions to the type. Then in 1923 appeared the first variant, a sport named *pointed tips* which later was found to be a haploid, presumably from the parthenogenetic development of a *franciscana* egg.

Plants of pointed tips, text-figure 2, are about half the stature of *franciscana* and with all organs proportionally smaller. The leaves are generally narrower and more sharply pointed and the very red bud cones are more attenuate. The flowers are about half the size of the parent species. Pollen is developed only in small amounts and frequently flowers will produce none at all, the anthers being shriveled. Some plants have

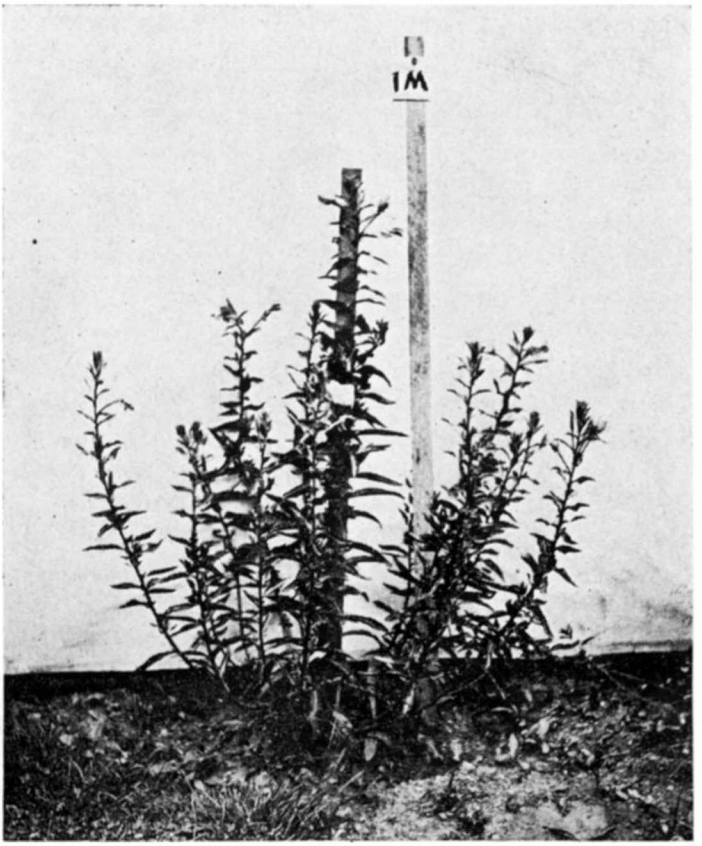


FIGURE 1.—*Oenothera franciscana* early in the flowering season. The central shoot may finally become more than 1 m. high.

been found to develop no pollen over several weeks of daily observation. In such pollen as may be formed, text figure 3B, frequently 60 to 90 percent of the grains will be shrunken. Since the pollen is so highly sterile few seeds are developed on selfed plants and the capsules are thin.

The first plant of pointed tips (23.21-165) was selfed and also backcrossed to the parent line of *franciscana*. The progeny of selfed lines is

almost wholly plants of the parent type, *franciscana*, but a few plants of pointed tips appear. The data of the selfed lines are given in table 1 and it should be noted that cultures 26.25 and 26.26 are second generations and that cultures 27.41, 27.42 and 27.43 are third generations. These selfed lines gave a total of 694 *franciscana* and 29 pointed tips. The behavior of the haploid when selfed was then what would be expected. Its fertile gametes carry the haploid set of *franciscana* chromosomes and their

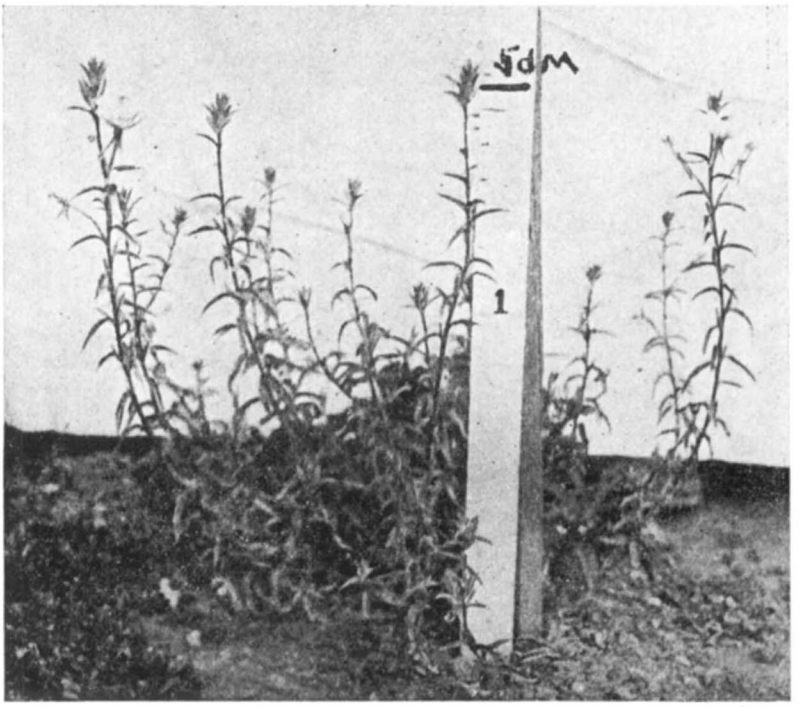


FIGURE 2.—The haploid, pointed tips, a plant of about one half the stature of *Oenothera franciscana*.

fusion would give the diploid zygote of *franciscana*. Such plants of pointed tips as appear are to be considered as coming from eggs that develop parthenogenetically. Of the pointed tips, 22 plants in one line (26.26 and 27.43) presented somewhat broader leaves than the type and a higher degree of pollen fertility; this form has not yet been studied cytologically and may possibly be a modification of the original haploid. A very interesting form, *red elongate*, which appeared in certain cultures, table I, will not be discussed at this time.

In addition to the first plant of pointed tips (23.21-165) there have appeared from later cultures of *franciscana* 3 more plants:—27.21-1108 from a culture numbering 1117 plants, 28.21-1 from a culture of 945 plants, and 28.61-813 from a culture of 847 plants. The total number of haploids so far thrown by *franciscana* has been 4 plants in thirteen genera-

TABLE 1

*Progeny in selfed lines from the first plant of pointed tips (23.21-165).*

| CULTURE | PARENT POINTED TIPS SELFED | PERCENT OF GERMINATION | SEEDLINGS | FRANCISCANA | POINTED TIPS | RED ELONGATE | FAILED TO MATURE | DIED |
|---|---|---|---|---|---|---|---|---|
| 24.25 1st generation | 23.21-165 | 94 | 361 | 329 | 24.25-248<br>24.25-332 | | 1 dwarf similar to pointed tips | 29 |
| 26.25 2nd generation | 24.25-248 | 74 | 37 | 29 | 26.25-13<br>26.25-14<br>26.25-20 | | | 5 |
| 26.26 2nd generation | 24.25-332 | 68.2 | 60 | 55 | 26.26-40 somewhat broader leaved | | 2 narrow-leaved rosettes | 2 |
| 27.41 3d generation | 26.25-13 | 75 | 138 | 107 | 27.41-82<br>27.41-130 | 13 | 1 large thick-leavedrosette<br>8 narrow-leaved rosettes | 7 |
| 27.42 3d generation | 26.25-14 | 25.7 | 51 | 39 | | 3 | 1 narrow-leaved rosette | 8 |
| 27.43 3d generation | 26.26-40 | 53.4 | 164 | 135 | 21 plants somewhat broader leaved | 1 | 1 large thick-leaved rosette | 6 |
| Total | | | 811 | 694 | 29 | 17 | 14 | 57 |

tions that have totaled 5690 plants. However, some of these generations have been small and did not express the results of complete seed germination so that the ratio of pointed tips out of *franciscana* is undoubtedly higher than these figures might suggest. Since the cultures 27.21, 28.21, and 28.61 were from seeds forced to complete germination and gave 3

pointed tips in a total of 2909 plants, it seems probable that the ratio of the haploid to *franciscana* is about 1:1000.

Pointed tips has also appeared in some crosses of *franciscana* to certain derivatives upon which the senior author is making studies on the inheritance of the *sulfurea* color and of dwarfness. The following three crosses threw each a single plant of the haploid:—culture 26.29 *franciscana*×*franciscana sulfurea*, 49 plants, gave pointed tips 26.29-6; culture 26.58 *franciscana*×(*franciscana*×*franciscana sulfurea* dwarf), 254 plants, gave pointed tips 26.58-103; culture 26.61 (*franciscana sulfurea* dwarf× *franciscana*)×*franciscana*, 214 plants, gave pointed tips 26.61-84. It is clear that pointed tips in cultures 26.29 and 26.58 might have come from the parthenogenetic development of *franciscana* eggs. Culture 26.61 must be briefly explained: its female parent was the hybrid *franciscana sulfurea* dwarf (*yytt*)×*franciscana* (*YYTT*); *Y* stands for yellow petals and *T* for normal height, absence of color (sulfurea) and dwarfness being recessive. The hybrid has the constitution *YyTt* and forms gametes *YT*, *Yt*, *yT* and *yt*. The egg *YT* has the chromosome set of *franciscana* and its parthenogenetic development would give pointed tips. The progeny of the three plants of pointed tips is given in table 2. As would be

TABLE 2

*Progeny from pointed tips out of crosses involving franciscana (f), franciscana sulfurea (fs), and franciscana sulfurea dwarf (fsd).*

| CULTURE | PARENT POINTED TIPS SELFED | PARENT CROSS | PERCENT OF GERMINATION | SEEDLINGS | FRANCISCANA | POINTED TIPS | OTHER FORMS | FAILED TO MATURE | DIED |
|---|---|---|---|---|---|---|---|---|---|
| 27.44 | 26.29–6 | *f*×*fs* | 28.7 | 277 | 249 | none | 8 broad-leaved dwarfs<br>3 thick, broad leaves | 6 weak rosettes | 11 |
| 27.45 | 26.58–103 | *f*×*fsd* | 39.2 | 126 | 115 | 27.45–112 | 2 light green<br>1 thick, broad-leaved rosette | 3 weak rosettes | 4 |
| 27.46 | 26.61–84 | (*fsd*×*f*)×*f* | 45.1 | 60 | 53 | none | 1 broad-leaved dwarf | 1 weak rosette<br>1 narrow-leaved dwarf | 4 |
| Total | | | | 463 | 417 | 1 | 15 | 11 | 19 |

expected almost all of the plants were *franciscana*. Only one pointed tips appeared, but there were several exceptional plants the most inter-, esting of which were *light green*, a new type in the lineage of *franciscana* and certain large, thick-leaved forms not yet fully studied.

The progeny of the back-crosses of pointed tips to the parent *franciscana*, table 3, was a total of 531 plants of *franciscana* and 3 dwarfs that failed to mature; there were no pointed tips. The *franciscana* plants were to be expected since the haploid gametes of pointed tips when formed

TABLE 3

*Backcrosses of the first plant of pointed tips to the parent species franciscana.*

| CULTURE | BACKCROSS | PERCENT OF GERMINATION | SEEDLINGS | FRANCISCANA | FAILED TO MATURE | DIED |
|---|---|---|---|---|---|---|
| 24.26 | franciscana×pointed tips 23.21-169×23.21-165 | 78.9 | 244 | 234 | 2 dwarfs | 8 |
| 24.27 | pointed tips×franciscana 23.21-165×23.21-169 | 83.7 | 298 | 297 | 1 dwarf | |
| Total | | | 542 | 531 | 3 | 8 |

have the same set of chromosomes as that carried by *franciscana* and their union with *franciscana* gametes would give the latter plant. If plants of pointed tips had appeared they would be interpreted as the result of parthenogenetic development of eggs whether from *franciscana* or from pointed tips.

It is appropriate to this paper to record the appearance of pointed tips in a large culture of the so-called *Oenothera Hookeri* of genetical literature. This plant is believed by BARTLETT not to be *Oenothera Hookeri* Torrey and Gray, the type specimen of which has marked canescent pubescence and is perhaps related to *Oenothera venusta* Bartlett. However, for the present it seems best to use the name *Hookeri* for the material first studied by DE VRIES and later by RENNER and others. The material of *Hookeri* is very closely related to *Oenothera franciscana* Bartlett and for purposes of field study might be considered a form of this species. *Hookeri* is not so sturdy a plant but it has the habit, pubescence and bud tips of *franciscana*, differing chiefly in its narrower leaves, bright red stems, red hypanthia, and in having more red on the bud cones. As in *franciscana* the pollen of *Hookeri* is almost wholly perfect and the seed fertility is very high (about 96 percent). *Hookeri* like *franciscana* has pairing chromosomes and is one of the few established homozygous species of Oenothera.

The line of *Hookeri* came from seeds kindly supplied by RENNER. Three generations have been grown:—culture 24.18 matured 202 plants, uniform; culture 27.26 matured 526 plants, uniform except for the plant of pointed tips (27.26-475); cultures 28.26 matured 563 plants, uniform.

Pointed tips from *Hookeri* has then appeared only once in a line of cultures totaling 1291 plants, a frequency similar to that in *franciscana* where the ratio has been about 1:1000. This plant (27.26-475) was typical pointed tips but wholly sterile as observed for 38 days during the flowering season. The flowers over this period produced only shriveled anthers.

In concluding the genetical account of this haploid Oenothera it should be emphasized that in every case the haploid might have arisen through the parthenogenetic development of an egg thus supporting the evidence so far reported for this manner of origin of haploids in plants.

## CYTOLOGY OF THE HAPLOID

The material for this cytological study came from the anthers of pointed tips 27.21-1108, the second plant of pointed tips to appear in the direct line of *franciscana*. The best results were from material fixed in the following modifications of Bouin's fluid: Sat. aq. sol. of picric acid 75 cc, commercial formalin 25 cc, glacial acetic acid 5 cc, chromic acid 1 g, urea 2 g. The anthers were left 5 1/2 hours in the fixing fluid, then rinsed in water and run through low grades of alcohol beginning with 2 1/2 percent, thirty minutes in each grade. Strong Flemming also gave satisfactory fixation; weak Flemming proved worthless. Sections were cut about 10$\mu$ and stained with iron alum haematoxylin.

The haploid count of 7 chromosomes was obtained in tissues of the developing flower. It is shown in plate 1, figure 1, which is the metaphase of a tapetal cell viewed from the pole.

There will first be described the history, illustrated in figures 2-29, which results in the formation of the very few good pollen grains that carry the normal set of 7 chromosomes. The resting nucleus of the microsporocyte, figure 2, contains a delicate chromatic network with numerous deeply staining bodies distributed over the periphery. As prophase comes on, figure 3, there takes place a gradual shortening and condensation of these strands which later, figure 4, give a coarse reticulum with threads of uneven thickness. The threads of this haploid reticulum are distinctly wider than the threads of the diploid *franciscana* at corresponding stages as shown in the excellent figures of CLELAND (1922, figures 1-4).

The stage of synizesis is clearly marked. It begins with the usual apparent contraction of the reticulum, figure 5, the threads showing irregular thickening. The point of greatest condensation with the contracted reticulum against the flattened nucleolus, figure 6, gives a picture similar to that of *franciscana* except that the threads are not so delicate and the

thread system seems to be not so long (see CLELAND 1922, figures 5-8). The emergence from synizesis, figure 7, through the apparent expansion of loops also follows the usual history in Oenotherae.

Out of synizesis comes the stage of the open spireme, figures 8 and 9, with the thickened threads rather evenly distributed through the nucleus. It becomes evident that the thread system is not so extensive as that in the diploid nucleus of *franciscana* at the corresponding stage. This is shown by a comparison of figures 8 and 9 with figures 14 and 15 given by CLELAND (1922) for *franciscana*.

The stage of second contraction is as clearly marked as in *franciscana*. The chromatic threads thicken and gather towards the center of the nucleus, figure 10. At this time it is evident that regions of the spireme are to become chromosomes, figure 11, although these structures are very closely grouped. With the loosening of the contracted chromatic mass, figures 12 and 13, the 7 chromosomes of this haploid set appear clearly outlined and the period of the second contraction comes to an end.

Then follows the stage corresponding to the characteristic period of diakinesis in diploid plants, but as would be expected there is here no pairing of chromosomes. The 7 chromosomes become irregularly distributed through the nuclear area as shown in figures 14–18. Occasionally two chromosomes may be found attached end to end by a delicate thread, plate II, figures 14 and 15, which possibly represents a portion of the original thread system still persisting.

The nucleus now makes an attempt to carry through a division at this period of the heterotypic mitosis in diploid plants. A multipolar spindle is shown in figure 19, but this fails to pass into the normal bipolar structure in the material which we are now considering. One pole may develop more or less clearly, figures 20 and 21, but the second pole is not formed, the spindle fibers ending over a broad and vague area. The 7 chromosomes move somewhat towards the single pole and then gather in a group and a nuclear membrane is formed around them, figure 22. This gives a nucleus in the period corresponding to interkinesis of normal meiosis but there has been no segregation of chromosomes, no heterotypic mitosis.

At this time the chromosomes divide lengthwise, and the nucleus then contains 7 split chromosomes, figure 23, such as are present at interkinesis in *franciscana*. As in *franciscana* the split does not appear until rather late in this period that corresponds to interkinesis.

Then comes the homoeotypic mitosis which is to separate the halves of the split chromosomes. Its metaphase, figure 24, with the 7 pairs of daughter chromosomes, now much condensed, is similar to the homoeo-

## Explanation of Plates

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 mm (num. aper. 1.30) in combination with the compensating ocular K 20×. The approximate magnification for figures 1–29 is 2400 diam.; for figures 30–37 it is 1800 diam.

Figures 1–29 give the history which results in the formation of a small proportion of the pollen grains with a normal set of seven chromosomes.

### Plate 1

Figure 1.—Metaphase of the mitosis in a tapetal cell as seen from the pole of the spindle, 7 chromosomes.

Figure 2.—Resting nucleus of the microsporocyte. Many deeply staining chromatic bodies appear near the periphery of the nucleus.

Figure 3.—Gradual thickening of the threads, some are still slender.

Figure 4.—A later stage in the process of thickening giving a coarse system of uneven threads.

Figure 5.—Early synizesis showing the contraction of the thread system.

Figure 6.—Mid-synizesis. Most of the threads are in closely twisted coils at the side of the large and rather flattened nucleolus.

Figure 7.—Late synizesis. The threads more loose, uniform and thicker.

Figure 8.—Open spireme. The threads show bead-like structures, probably chromomeres.

Figure 9.—A later stage of the open spireme with threads more uniform in thickness.

Figure 10.—Second contraction. Note the central thickening of the threads and the peripheral loops.

Figure 11.—Late second contraction. Chromosomes distinct but much crowded.

Figure 12.—End of the second contraction. The 7 chromosomes are still clustered around the large nucleolus.

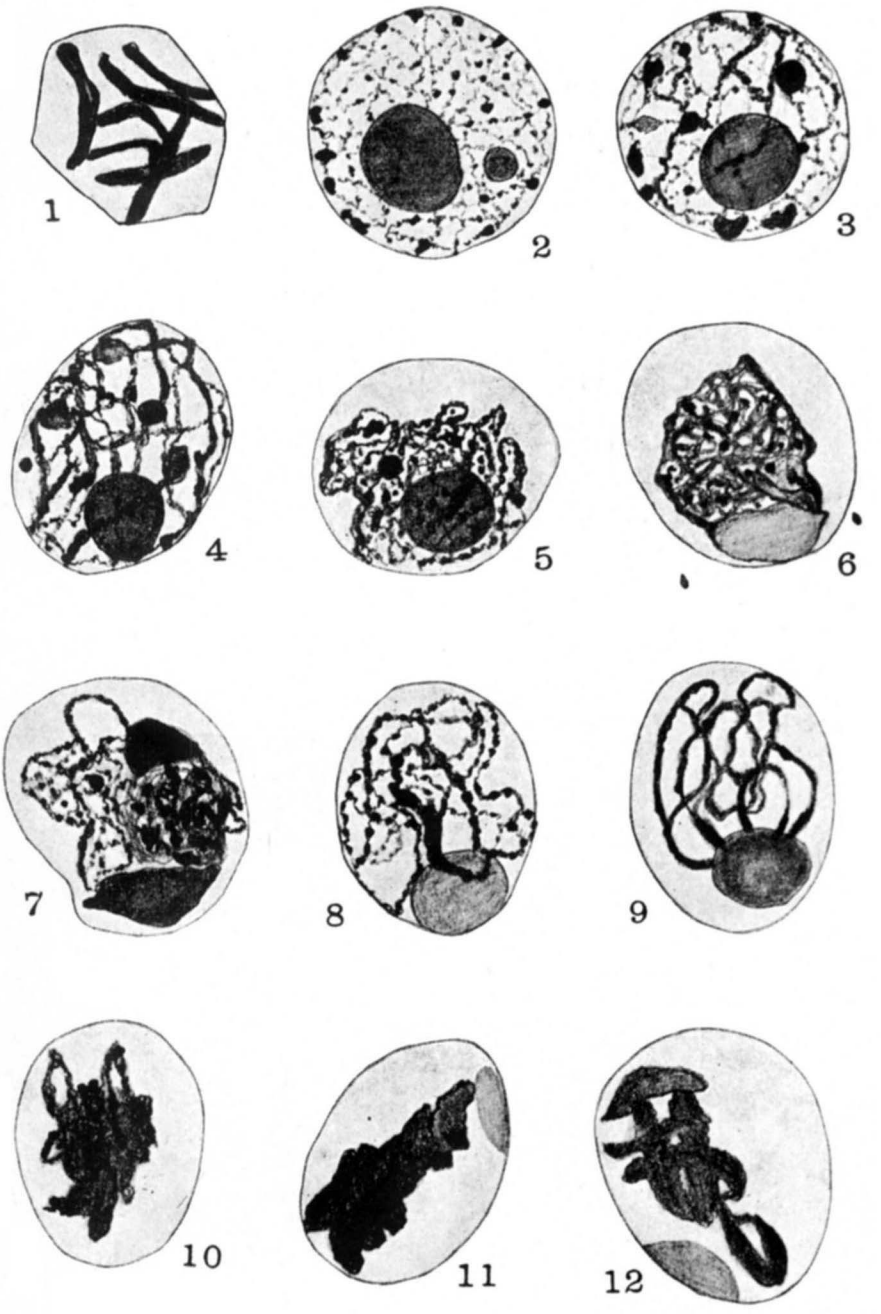
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PLATE 2

FIGURE 13.—Chromosomes shortly after their emergence from second contraction.

FIGURES 14, 15, 16, 17, 18.—Late prophase corresponding to diakinesis. The 7 chromosomes distinct.

FIGURE 19.—Multipolar spindle. The 7 chromosomes gathered in the center of the spindle.

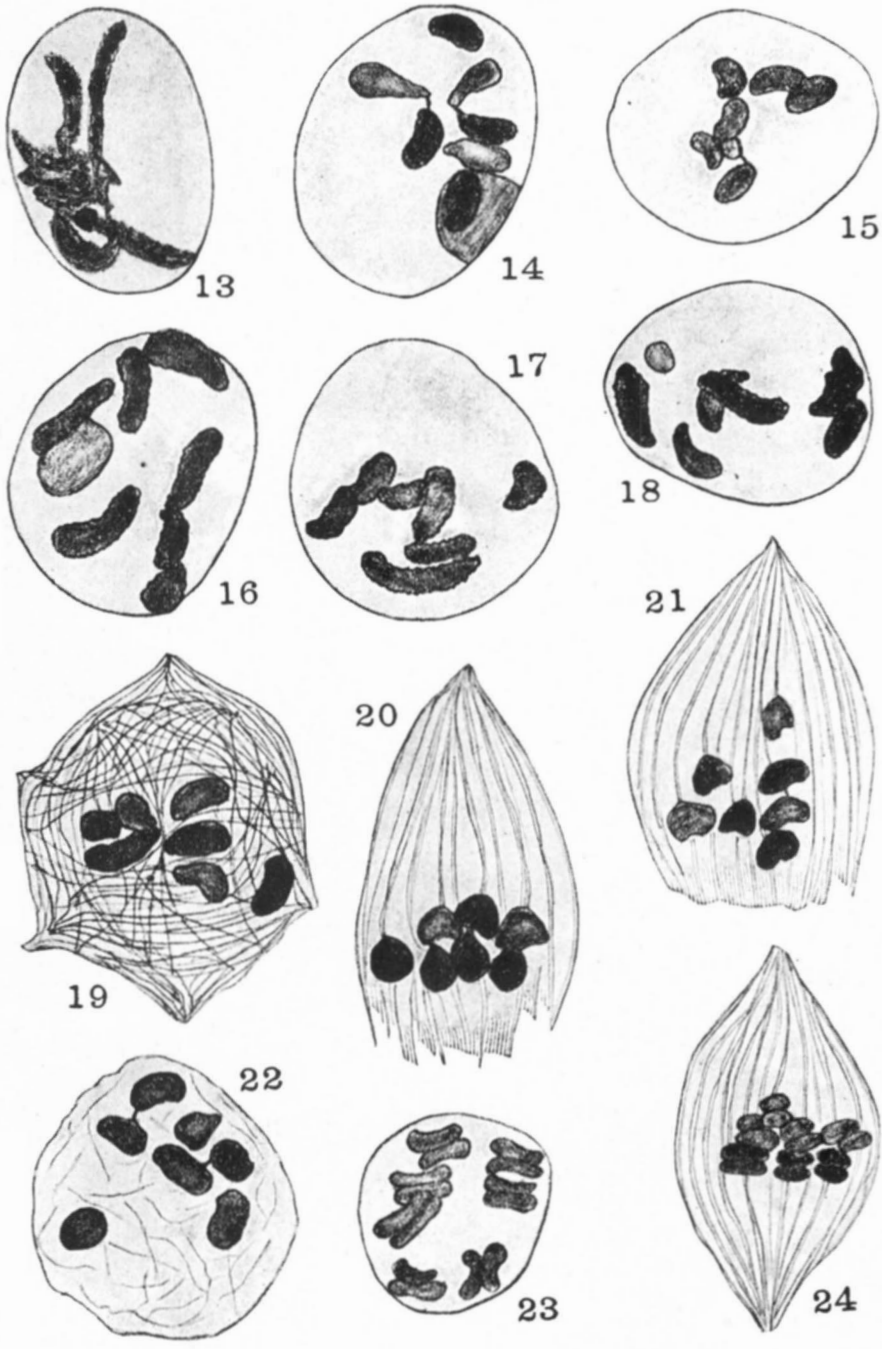
FIGURE 20.—Unipolar spindle. The 7 chromosomes in a plate arrangement.

FIGURE 21.—Unipolar spindle. The 7 chromosomes moving somewhat towards the pole.

FIGURE 22.—Reconstituted nucleus following non-reduction, 7 chromosomes.

FIGURE 23.—Reconstituted nucleus in stage corresponding to interkinesis, 7 split chromosomes.

FIGURE 24.—Metaphase of the homoeotypic mitosis, 7 split chromosomes at the equatorial plate.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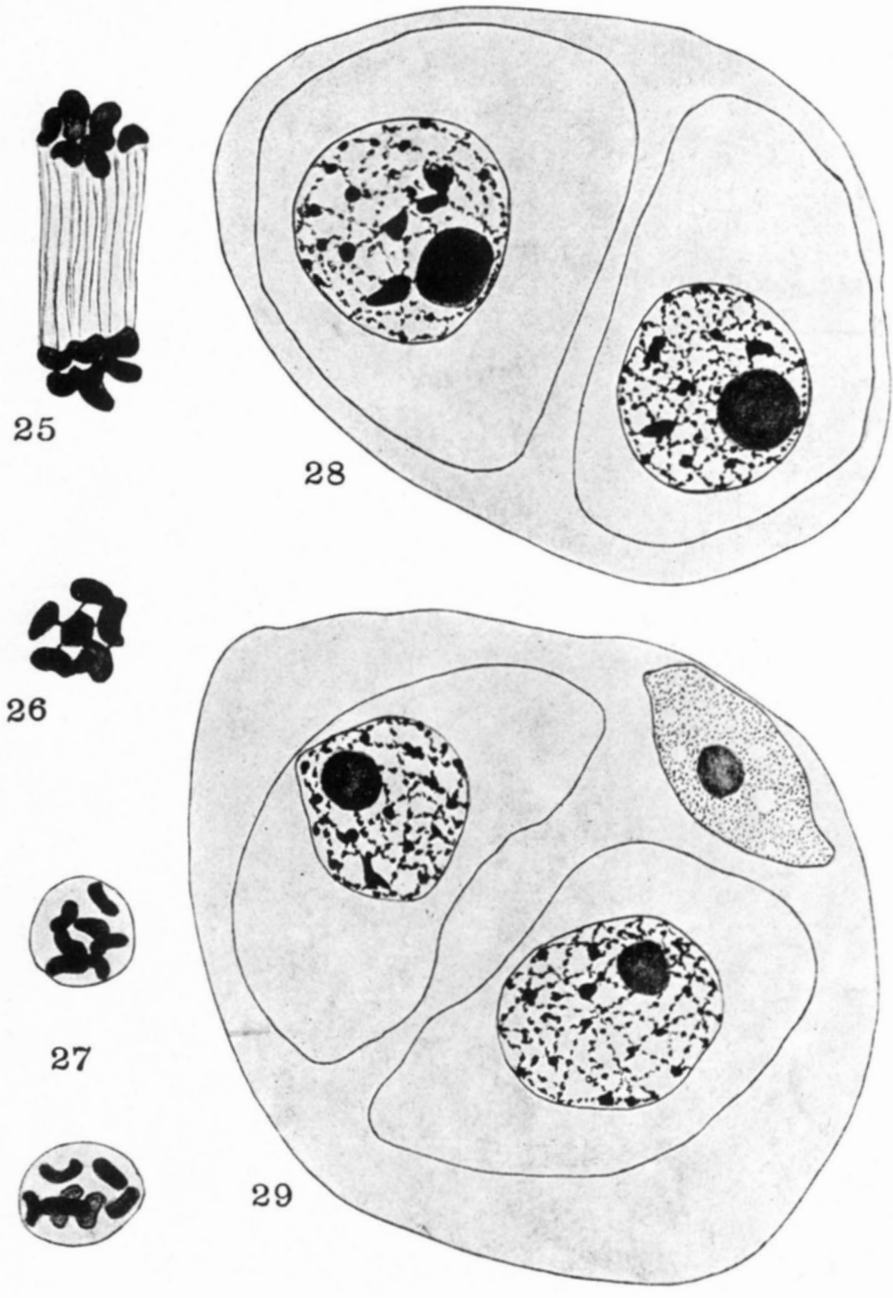
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PLATE 4

Figures 30–35 show irregular distribution of chromosomes during meiosis which will result in shrivelled pollen grains.

FIGURE 30.—Heterotypic anaphase; 6 chromosomes passing to one pole and 1 to the other.

FIGURE 31.—Late heterotypic anaphase; 5 chromosomes at one pole and 2 at the other.

FIGURE 32.—Heterotypic anaphase; 5 chromosomes passing to one pole and 2 to the other.

FIGURE 33.—Heterotypic anaphase; 4 chromosomes passing to one pole and 3 to the other.

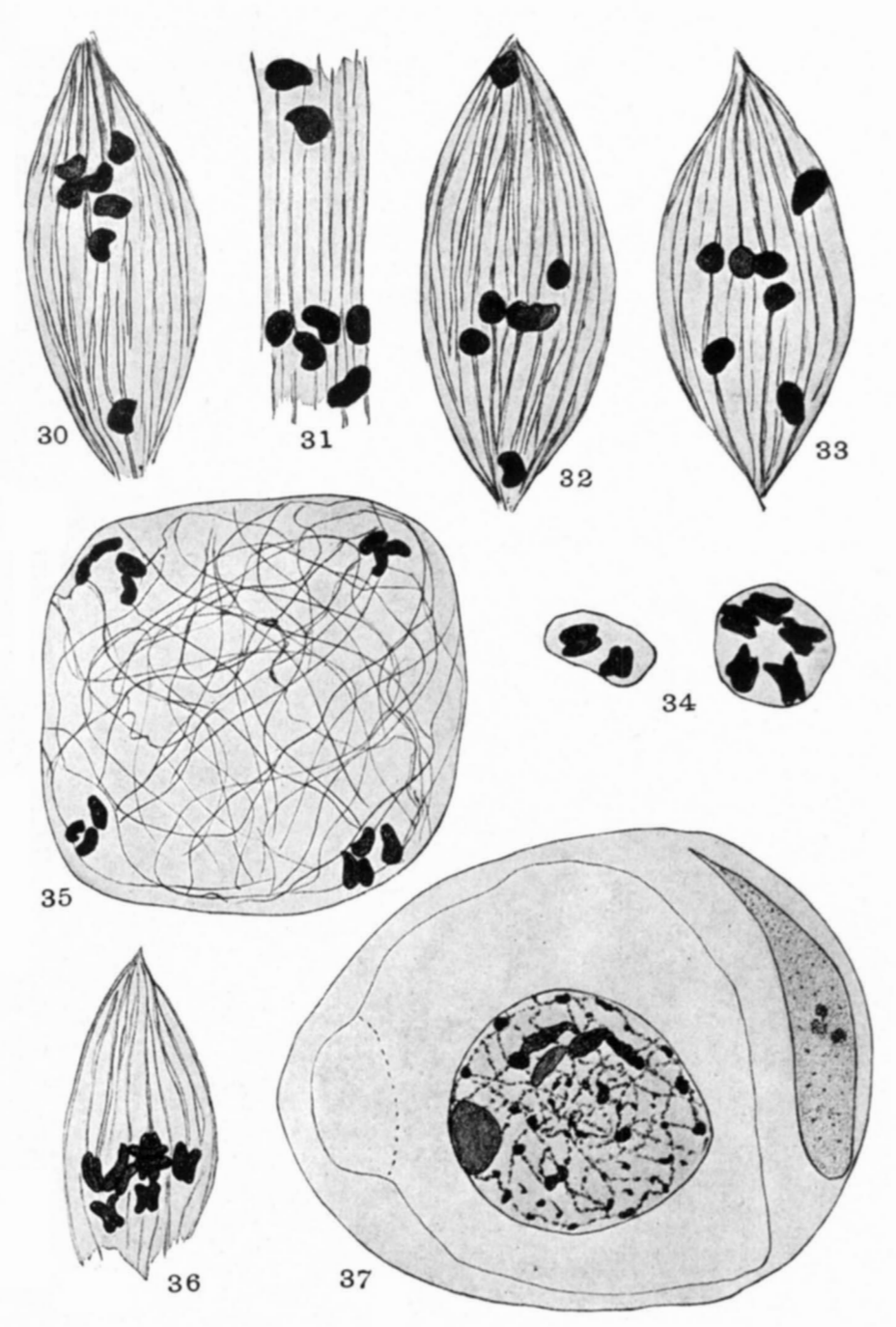
FIGURE 34.—Interkinesis with split chromosomes; five bivalents in one nucleus and two in the other.

FIGURE 35.—Homoeotypic anaphase showing irregular distribution of the 14 chromosomes, two nuclei with 3 and two nuclei with 4 chromosomes.

Figures 36 and 37 show the possible formation of giant pollen grains with 14 chromosomes, a double haploid set.

FIGURE 36.—A unipolar spindle in the homoeotypic mitosis following non-reduction in the heterotypic; 7 split chromosomes, remaining together, will produce a nucleus with 14 chromosomes.

FIGURE 37.—A giant pollen cell accompanied by a mass of pinched off cytoplasm. Such a cell will possibly produce a giant pollen grain with 14 chromosomes.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typic metaphase of *franciscana*, but there is only one spindle in the sporocyte. Anaphase follows, plate 3, figure 25, and the sets of daughter chromosomes move to the poles, figure 26, to organize two daughter nuclei, figure 27, each with 7 chromosomes.

The two nuclei enlarge, pass into a resting condition, the cytoplasm divides, figure 28, and two pollen grains are developed within the microsporocyte. Figure 29 shows the same result with the complication that a small mass of cytoplasm has separated from the two daughter cells. MATSUDA (1928) reports for Petunia similar divisions leading to small masses of cytoplasm without nuclei. These pollen grains are haploid and

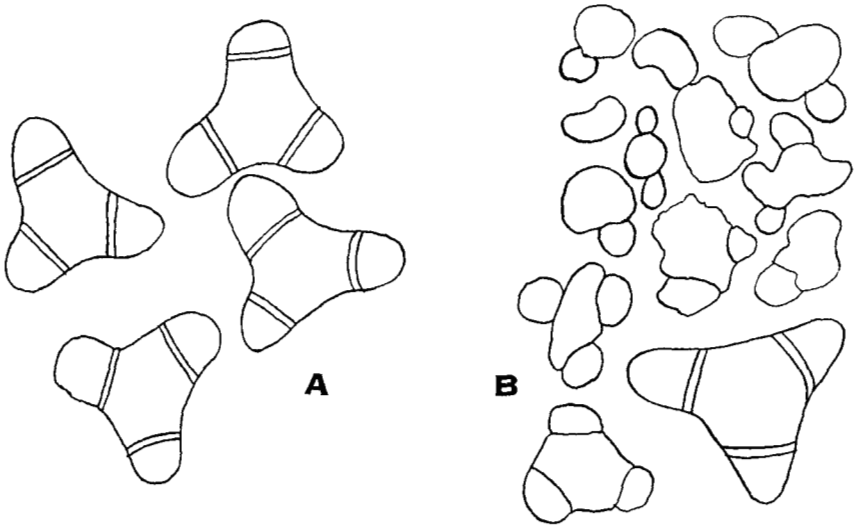


FIGURE 3.—A. Pollen of *Oenothera franciscana*. B. Pollen of the haploid, pointed tips; one good grain is shown among shriveled examples.

they are believed to constitute the fertile pollen which produces *franciscana* zygotes when pointed tips is selfed and when *franciscana* is pollinated by pointed tips. These good pollen grains are of the same size as those of *franciscana*, text figure 3B.

Most of the pollen grains, when produced at all by pointed tips, are shriveled, text figure 3B. Such samplings and estimates as have been made indicate that rarely more than 10 percent of the pollen appears normal in the small amount that an anther may produce. The shriveled grains are to be explained through irregular distribution of chromosomes during meiosis giving grains with less than 7 chromosomes as illustrated

in the series of plate 4 figures, 30–35, which will now be described. In pointed tips there is of course very much more of this material than that which results in pollen grains with the haploid set of 7 chromosomes.

The irregularities of distribution come during the heterotypic mitosis when bipolar spindles are formed and the 7 chromosomes are separated in the possible assortments of 1 and 6,2 and 5,3 and 4. All of these possible results of segregation have been observed and they are illustrated in figures 30–33; the attachments of spindle fibers show the direction of chromosome movement. Figure 30 presents the distribution of 1 and 6, figures 31 and 32 the distribution of 2 and 5, and figure 33 the distribution of 3 and 4.

The results of these divisions are microsporocytes containing a larger and a smaller nucleus. Figure 34 illustrates a case following a segregation of chromosomes, 2 and 5. During the interkinesis the chromosomes split lengthwise, figure 34, and the daughter chromosomes are distributed in the usual way by the homoeotypic mitosis, figure 35, which generally ends with the formation of 4 cells that become abortive pollen grains.

Of particular interest in this material of pointed tips was the discovery of behavior which might lead to the formation of giant pollen grains with 14 chromosomes, a double haploid set. In figure 36 is shown a unipolar homoeotypic spindle with its 7 split chromosomes. This condition must have come from a one-nucleate sporocyte following a suppressed heterotypic mitosis, after which the chromosomes had split as illustrated in figure 23. The condition then followed a unipolar heterotypic spindle such as is shown in figures 20 and 21. Such a failure of the homoeotypic mitosis to distribute the halves of the 7 split chromosomes would result in a giant cell with 14 chromosomes which might become a pollen grain. A single example of such a cell was observed, figure 37, and in this case a small mass of cytoplasm had been pinched off at one side presenting behavior similar to that shown in figure 29. Through such a history might arise in haploid plants from time to time giant pollen grains produced singly in the microsporocytes and carrying two haploid sets of chromosomes. A triploid plant would result through the fertilization of a haploid egg from such a pollen grain and a tetraploid plant through the fertilization of an egg of like constitution.

## DISCUSSION

The cytology of the Datura haploids has been described by BELLING and BLAKESLEE (1923, 1927). The 12 chromosomes show no attraction for one another at metaphase of the heterotypic mitosis in the micro-

sporocytes but either move at random to the poles (in assortments of 1 and 11, 2 and 10,3 and 9,4 and 8,5 and 7,6 and 6), or there is non-reduction. When there is segregation the groups of chromosomes after a short interphase pass through a homoeotypic mitosis where each divides and the halves are distributed in the usual manner. Such microsporocytes form 4 small microspores, usually 2 equal and smaller and 2 equal and larger. Polyspores are developed when irregularities during anaphase of the first division give chromosomes independent of the main groups and these organize very small cells in addition to the 4 principal cells. All of these small grains constitute the mass of the abortive pollen which is developed. Non-reduction takes place when the 12 chromosomes split and the halves are distributed in two sets, 12+12, and two pollen grains result each with the haploid set of chromosomes. These constitute the good pollen grains which make up about 12 percent of the total product. The good pollen grains are about the same size as the grains from the diploid Datura. Haploids selfed give diploids as would be expected.

The haploid Oenothera appears to follow the same history as the haploid Datura. Its 7 chromosomes show the same irregular distribution by the heterotypic mitosis in assortments of 1 and 6,2 and 5,3 and 4. The good pollen grains are likewise found 2 in a microsporocyte as the result of one mitosis which distributes the halves of the 7 chromosomes which split. This mitosis in Oenothera is the homoeotypic division, the heterotypic mitosis being suppressed because a bipolar spindle is not developed. BELLING and BLAKESLEE do not take a clear position on the homology of the single mitosis in Datura whether heterotypic or homoeotypic. We suspect it to be the homoeotypic mitosis as in Oenothera and think that they have failed to recognize the suppression of the heterotypic. This might readily happen in studies on iron-acetocarmine preparations which do not show spindle structure.

CHIPMAN and GOODSPEED (1927) give an account of meiosis in the microsporocytes of the haploid from *Nicotiana tabacum* var. *purpurea*, one of the two haploids reported by CLAUSEN and MANN (1924). This haploid (24 chromosomes) was female sterile but some viable pollen is produced. There is a stage of synizesis followed by pachynema which segments into the haploid set of chromosomes which do not pair at diakinesis. Bipolar spindles are often formed resulting in a random distribution of the 24 chromosomes some of which occasionally pass into the cytoplasm. Univalents sometimes divide during the heterotypic mitosis and when rarely all 24 divide there results a giant spindle and the formation of diads which might develop into pollen grains with the full set of

haploid chromosomes. There was some evidence of the occasional suppression of the heterotypic spindle and the gathering of the haploid set of chromosomes into a single nucleus as occurs after the unipolar spindle of our haploid Oenothera. A normal homoeotypic mitosis following such behavior would give diads with the full set of haploid chromosomes. A peculiar feature of the Nicotiana haploid, as also of the haploid wheat, is the precocious division of the univalents at heterotypic metaphase which we have not found in Oenothera. In other respects there seems to be a fairly close agreement in meiotic behavior between the haploid Nicotiana and the haploid Oenothera.

GAINES and AASE (1926) in their account of the haploid wheat were the first to publish a detailed description of the cytology of a haploid spermatophyte. The haploid (21 chromosomes) came from seed of a hybrid wheat No. 128, *Triticum compactum humboldtii* Kcke. (42 chromosomes) after an attempt to pollinate by *Aegilops cylindrica*. The haploid was not to be distinguished from the female parent No. 128 until the time of flowering when peculiarities characteristic of sterility appeared. The plant was about 99.8 percent seed sterile. There was no pairing of chromosomes during meiosis in the microsporocytes because there were probably three dissimilar sets of 7 chromosomes each. The chromosomes are generally distributed irregularly during the heterotypic mitosis but sometimes the chromosomes divide and the halves pass in an orderly manner to opposite poles, simulating a homoeotypic mitosis, or there may be a mixture of the two processes. The homoeotypic mitosis continues the disorderly distribution of the chromosomes giving many irregularities and forms of polyspory. No normal pollen grains were observed to develop although such might rarely be formed. An uncommon peculiarity was observed in the fusion of adjacent microsporocytes after which giant spindles may be developed including the chromosomes of two or more nuclei; these do not pair and are distributed at random. This would seem to be behavior similar to the process of "endo-duplication" described by JÖRGENSEN (1928). From such fusions come giant pollen grains which may occasionally be found in the mixture of abnormalities produced. Except for the extraordinary fusions of the microsporocytes and the early division of the chromosomes before their distribution by the heterotypic mitosis most of the irregularities of this haploid wheat are present in our haploid Oenothera which in addition gave us the history of the small amount of fertile pollen produced.

Very different in some important respects from the other cytological accounts of haploids is the interesting description of some Solanum

haploids by JÖRGENSEN (1928). The pollen tube of *Solanum luteum* may enter the embryo sac of *S. nigrum* and discharge its two sperm nuclei but these fail to fuse with the egg and endosperm nucleus and finally disintegrate. From some of the unfertilized eggs embryos begin to develop and there can scarcely be doubt that the seeds which produce haploids come from such parthenogenetic development. During meiosis in the microsporocyte the 36 chromosomes of the haploid at diakinesis show some degree of pairing, the number of pairs ranging from 3 to 11 or 12. The larger number of pairs suggests a reduction division of the $12_2+12_1$ type. At the heterotypic metaphase only the bivalents are constantly present on the equatorial plate, the univalents being scattered through the cell and most often in the polar regions. The number of bivalents on the plate ranges from 3 to 12 with the numbers 5 to 8 most frequent. Univalent chromosomes lying near the plate may divide and their products can be recognized at the homoeotypic metaphase by their small size. The chromosomes pass irregularly to the poles in numbers ranging from 15 to 22 with 18 most frequent. The homoeotypic mitosis more commonly presents 18 chromosomes at metaphase; these divide and anaphase proceeds regularly giving 4 pollen grains each as a rule with 18 chromosomes. Meiosis in the megasporocyte is peculiar in that more of the chromosomes during the heterotypic mitosis pass to the micropylar end of the cell. Since the embryo sac develops from the chalazal megaspore it receives a nucleus with the smaller number of chromosomes (14 to 16) which apparently are not enough for further development since the nucellus soon breaks down. A striking peculiarity of the Solanum haploids is the pairing of certain chromosomes in the heterotypic mitosis. This, as JÖRGENSEN points out, suggests that the 36 chromosomes of the haploid constitute a group composed of 3 sets of 12 each of which 2 sets contain homologues of sufficient similarity to bring them together in a true synapsis. Such behavior would not be expected and does not occur in Datura, Nicotiana or Oenothera where the haploid count is the basic chromosome number of the genus.

The most interesting feature of the process leading to the development of the fertile pollen of the haploid Oenothera is the clear cut suppression of the heterotypic mitosis through failure to develop a normal spindle with the result that a reconstituted nucleus takes the seven chromosomes which then divide and the halves are distributed by a homoeotypic mitosis to form two pollen grains in each microsporocyte. There is then no reduction division and the fertile pollen carries the somatic set of seven chromosomes characteristic of the haploid. ROSENBERG (1917) first de-

scribed such a history for *Hieracium laevigatum* and *H. lacerum* and called it a "semi-heterotypic" division including under the term also the cases where chromosomes without conjugation are distributed irregularly by the heterotypic mitosis to give diads with various chromosome counts. This latter behavior is also found in the haploid Oenothera and gives the large mass of shriveled pollen grains present.

The term "semi-heterotypic" does not seem to us fortunate since the heterotypic mitosis either fails entirely to operate or performs irregularly; in neither case is it a half expressed division. We prefer to call the behavior of the first type suppression of the heterotypic mitosis and it is of course a very important modification of sporogenesis since it results in fertile pollen with the set of chromosomes characteristic of the parent. ROSENBERG was quick to point out that suppression of the heterotypic mitosis permits the doubling of chromosome numbers through the union of gametes carrying the somatic sets of the parents. Thus, as from fertile haploids come diploids so from diploids might come tetraploids. His earlier work was on parthenogenetic Hieracia but in later papers (ROSENBERG 1926, 1927) he develops this idea and suggests the possible origin of tetraploid hybrids through the union of gametes produced with nonreduction.

Material support for ROSENBERG'S views on the origin of tetraploid hybrids has appeared in the interesting cross of Raphanus × Brassica recently described by KARPECHENKO (1927). The $F_1$ hybrids may form diploid pollen grains by the suppression of the heterotypic mitosis. Tetraploid pollen grains may arise through two steps:—(1) A tetraploid group is established by the association of two nuclei in a microsporocyte and the gathering of the two diploid sets of chromosomes through the fusion of the two heterotypic spindles; (2) The failure of the two combined heterotypic spindles to bring about segregation leads to a reconstituted nucleus with the tetraploid group, and tetraploid diads are formed following a homoeotypic mitosis. Unions of haploid, diploid and tetraploid gametes in various combinations, complicated by some numerical irregularities of chromosome distribution, give possibilities of a large assortment of polyploids which were in part realized. Among these the tetraploids from the union of diploid gametes were noteworthy as fertile plants showing no segregation and with characters of a species distinct from both parents. They show one way through which stable and fertile tetraploid hybrids may arise in sharp contrast to the "indirect chromosome binding" of WINGE (1917,1925), and the somatic tetraploidy by "endo-duplication" of JÖRGENSEN (1928). It is interesting to note how

complicated has become the subject of the origin of polyploids, with a number of possible methods probably present in different groups of plants.

Haploids carrying only non-homologous chromosomes have a peculiar interest in genetics because there can be no question of heterozygosity to complicate, if they are fertile, a study of their progeny. When selfed their seed should give homozygous diploids unless gene mutations take place or irregularities of chromosome distribution produce chromosomal sports which may be checked by cytological studies. Both chromosomal sports and gene mutations in diploids from haploids have been noted in Datura (BLAKESLEE and others 1927). Two gene mutations, curled and tri-carpel, have been established. They are rare and recessive and the evidence indicates that the mutations probably occur in the egg or pollen grain of the haploid parents. In the selfed lines from the first plant of pointed tips, table 1, an interesting plant, *red elongate*, with important distinguishing characters appeared in three cultures (27.41, 27.42 and 27.43). One family (28.35) has been grown from red elongate selfed; it consisted of 185 *franciscana*, 20 red elongate, 1 pointed tip and 1 broad-leaved dwarf. The result suggests irregularities of chromosome distribution and offers an interesting subject for study. Progeny from another selfed plant of pointed tips out of the cross *franciscana*×*franciscana sulfurea* dwarf, table 3 (culture 27.45), gave two remarkable plants of a wholly new type named *light green* which has pollen almost wholly perfect. From light green selfed one family (28.37) has been grown consisting of 113 plants all true to the parent type. This behavior suggests a gene mutation and it should be studied in contrast to red elongate.

## SUMMARY

1. A haploid sport, named pointed tips, is thrown by the homozygous *Oenothera franciscana*. The plants are of about half the stature of *franciscana* with all organs proportionally smaller. Pollen is produced in small amounts and frequently flowers develop none at all, the anthers becoming shriveled. The pollen contains from 60 to 90 percent of shrunken and empty grains. Some fertile ovules are developed and the plant when selfed sets a small amount of seed.

2. The haploid thus far has appeared only four times in a series of generations of *O. franciscana* in a ratio of about 1:1000. It has also been thrown from crosses of *franciscana* to certain derivatives.

3. Pointed tips selfed, tables 1 and 2, gives a progeny almost wholly of the parent type *franciscana*, but a few plants of pointed tips appear and occasionally other forms. Of the latter, red elongate and light green merit attention and further study.

4. Pointed tips backcrossed to *franciscana*, table 3, gave only *franciscana* but in large cultures an occasional haploid would be expected.

5. Pointed tips is also recorded from a large culture of *Oenothera Hookeri*, a homozygous form very closely related to *O. franciscana*.

6. In all cases the haploid may be interpreted as arising from the parthenogenetic development of an egg. This interpretation finds support in the fact that pointed tips selfed and backcrossed to *franciscana* gives almost wholly *franciscana* showing that its gametes must be of the *franciscana* genotype.

7. The few fertile pollen grains of the haploid are developed as the result of the suppression of the heterotypic mitosis. The prophases are as in *franciscana* except that there is no pairing of the 7 chromosomes at diakinesis. The heterotypic spindle fails to attain the bipolar condition although one pole is generally evident. The chromosomes are not distributed but remain in a group and a nucleus is reconstituted which corresponds to that in the period of interkinesis of normal meiosis. In this nucleus the chromosomes split exactly as in interkinesis. Then comes a homoeotypic mitosis which distributes the halves of the split chromosomes to give two nuclei with 7 chromosomes each, and two fertile pollen grains are developed in each microsporocyte.

8. The large mass of shriveled and sterile pollen grains results from the irregular distribution of the 7 chromosomes by the heterotypic mitosis in assortments of 1 and 6,2 and 5,3 and 4. In this manner the microsporocyte comes to contain a larger and a smaller nucleus neither of which has the full complement of 7 chromosomes. During interkinesis the chromosomes split and the halves are distributed as usual by a homoeotypic mitosis with the result that four nuclei are formed all lacking the full set of chromosomes. Cell division usually gives four small pollen grains which become shrunken and empty of contents.

9. Very rarely a homoeotypic spindle following the suppression of the heterotypic mitosis will itself fail to reach full development. In such cases the 7 split chromosomes of the period corresponding to interkinesis enter a reconstituted nucleus and a single giant pollen grain with 14 chromosomes may be produced by the microsporocyte.

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