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TITLE A Study of Seed Sterility in Crosses Between OENOTHERA FRANCISCANA BARTLETT And its Tetraploid

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A STUDY OF SEED STERILITY IN CROSSES BETWEEN *OENOTHERA FRANCISCANA* BARTLETT AND ITS TETRAPLOID

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan

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A STUDY OF SEED STERILITY IN CROSSES BETWEEN *OENOTHERA FRANCISCANA* BARTLETT AND ITS TETRAPLOID

INTRODUCTION

Oenothera has proved to be one of the most complicated and interesting genera in the fields of genetics and cytology. Very few of the many species studied have proved to be genetically pure, and among these is *Oenothera franciscana* Bartlett. This plant has been carried in selfed line by Prof. B. M. Davis since 1913 and it gave no variant until the tenth generation when the first haploid sport appeared, probably from the parthenogenetic development of a *franciscana* egg (Davis and Kulkarni 1930). From the cross *franciscana* ^{by} x *franciscana sulfurea nana* Davis secured four triploids in the F_2 generation and from one of these triploids he obtained the first tetraploid plant (Davis 1933).

The first cytological study on this material was that of Cleland (1922) on the diploid. This was followed by the report by Davis (1933) on the triploid and the tetraploid. These studies were made on the pollen mother cells. The megaspore mother cells were not studied and the present investigation was therefore directed to the megasporocytes of these three types.

Oenothera franciscana is about 90 per cent seed fertile and its tetraploid derivative about 60 per cent seed fertile, but the reciprocal crosses between the two have proved to be highly seed sterile. These peculiarities led to studies on developments within the ovules of these two crosses and their parents in an attempt to determine the cause of this sterility. The present paper therefore presents first, results from the studies on meiosis in the megasporocytes of the diploid franciscana and its tetraploid and triploid derivatives, and second, conclusions on the high seed sterility found in the reciprocal crosses between franciscana and the tetraploid.

MATERIAL AND METHODS

While in vials of water the seeds were subjected to alternate exhaust and pressure of 30 pounds three times during 24 hours. This treatment led to rapid germination after the seeds were placed on wet filter paper in Petri dishes. The germinating seeds were removed to other Petri dishes and after a few days the seedlings were large enough to be planted in pans of earth. Practically all of the viable seeds germinated within two weeks but those of franciscana germinated much more rapidly than the others. Later the young plants were transferred from the pans to small pots in which they were kept in the green house until near the end of May, at which time they were set out in the field.

While the plants were young rosettes in the green house, a classification was made and root tips from representatives of the various groups were fixed for chromosome counts. At this time photographs were taken of the main types in the different cultures. Text figure 1 shows young rosettes: A, the diploid; B, the tetraploid; C and D, triploid reciprocal crosses.



Text figure 1. Four young rosettes. A, Oenothera franciscana. B, tetraploid, with broader and thicker leaves. C, triploid, from the cross franciscana x tetraploid. D, triploid, from the cross tetraploid x franciscana.

Most of the cytological and all of the embryological material came from these plants.

The cytological material for studies on meiosis was collected from ovaries in various stages of development. Material for the problem of seed sterility was collected from ovaries following cross pollination between franciscana and the tetraploid. This latter material was fixed at the following times: 48, 72, 96, 120, 144 hours after pollination, and for later stages 7, 8, 9, 10, 15, 20 and 25 days after pollination, and finally the mature seeds.

The hairy outside of the ovary was cut away, and each ovary was then cut crosswise at points about 2 mm apart to facilitate the penetration of the fixing fluid. Collections were made largely between 10 and 12 o'clock in the morning and 1 and 3 o'clock in the afternoon. All the material was fixed in the field.

Experiments determined that fixation by Navashin's fluid, as modified by Karpechenko, was as good as that by Allen's modification of Bouin's fluid, and for greater convenience the former was

more frequently employed.

Navashin's fluid (Karpechenko's modification):

Solution A:	Chromic acid	4	grams
	Glacial acetic	40	cc
	Water	240	cc
Solution B:	40% Formaldehyde	160	cc
	Water	140	cc

Schedule of treatment:

1. Solution A and B in equal parts. 3-4 hours. (A and B solutions were mixed in the field).
2. Transferred to Solution A. 14-18 hours.
3. Washed in water. 24 hours or overnight.
4. Long series of alcohol, 13 changes. 30 minutes between changes in the first half of the series and one hour in the second half.
5. 95% alcohol. 24 hours.
6. Absolute alcohol. 12 hours with 2 changes.
7. Cleared through cedar oil, removed by xylol.
8. Imbedded in hard paraffin.

Sections of cytological material were cut 10-12 microns thick. All sections were stained in crystal violet, after the Gram method and usually by the

following schedule:

1. Crystal violet, 1% aqueous solution. 15 minutes.
2. Rinsed in distilled water.
3. Aqueous solution of iodine and potassium iodine. 3-5 minutes. (1 gram of each in 300 cc of water).
4. Rinsed in distilled water.
5. Passed through 35%, 50%, 70%, 85% and 95% alcohol.
6. Absolute alcohol.
7. Clove oil, by judgement.
8. Xylol through 2 wells. 20 minutes.
9. Canada balsam.

With this stain the chromosomes even when crowded are so translucent that they may usually be identified when they lie at different levels in the nucleus.

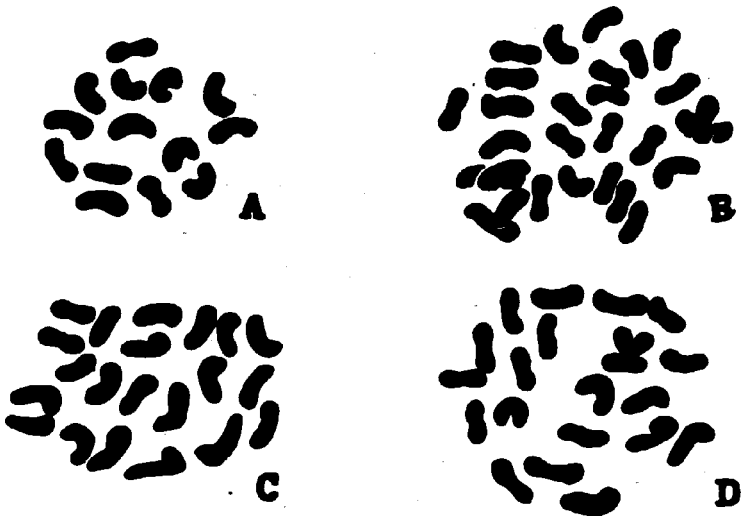
GENETICAL FEATURES OF *OENOTHERA FRANCISCANA*, THE TETRAPLOID, AND THE TRIPLOID

Oenothera franciscana

The diploid *franciscana* is well known in *Oenothera* research as a strong, vigorous plant, breeding very true except for occasional haploids. The pollen is almost wholly perfect and seed fertility is around 90 per cent. The present line has 7 pairs of chromosomes as tested by Davis in 1928, 1933 and 1935. The segregation of chromosomes during meiosis is highly regular. It is a remarkably stable species. A young rosette is shown in text figure 1, A, and the chromosome set from the root tip of this plant is given in text figure 2, A.

The Tetraploid

The tetraploid line arose by way of a triploid plant in the F_2 from the cross *franciscana* x *franciscana sulfurea nana*. *Franciscana* (SSDD) has yellow flowers while *franciscana sulfurea nana* (ssdd) has



Text figure 2. Chromosome counts from root tips of the four young rosettes shown in text figure 1. A, franciscana, 14 chromosomes. B, tetraploid, 28 chromosomes. C, triploid, 21 chromosomes. D, triploid, 21 chromosomes.

sulfur colored flowers and is about half as tall. The F_1 generation (SsDd) of this cross is uniformly like franciscana, yellow flowers and tall stature being dominant over sulfur flowers and dwarfness. The second generation gave the expected segregation of yellow talls, yellow dwarfs, sulfur talls and sulfur dwarfs. Among the yellow talls were four triploid plants with 21 chromosomes which must have arisen through the union of a 7 chromosome gamete with a gamete of 14 chromosomes. Since the triploid and the later derived tetraploid threw no segregates for the recessive characters, it was assumed that the 7 chromosome gamete was SD and the 14 chromosome gamete SDSD. The SD gamete being the normal franciscana gamete, should constitute one fourth of the gametes developed by the F_1 hybrid SsDd. The SDSD gamete could only have arisen through the suppression of the homoeotypic division of a SD 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 SDSD. In the progeny of the selfed triploid appeared the plant

which proved to be a tetraploid, 28 chromosomes. The tetraploid is assumed to have arisen from the union of two 14 chromosome gametes derived from the triploid, each SDSD, and therefore to carry four sets of franciscana chromosomes and for the two characters involved to have the formula SDSD-SDSD. All of this was reported by Davis (1933). The tetraploid plants are not taller than franciscana but are stouter in almost every respect, having thicker leaves and bud cones. The broader and thicker leaves become evident even in the seedlings and young rosettes. Some of these peculiarities are shown in text figure 1, B. The set of 28 chromosomes from this plant is illustrated in text figure 2, B. Through seven generations selfed the tetraploid has proved to be very stable with the average seed germination of 60 per cent. Its pollen is about 20 per cent good, the grains being four or more lobed.

The Triploid

There is given in table 1, page 14, three cultures from crosses between franciscana and the

tetraploid. Culture 35.53 was the F_1 of the tetraploid x franciscana. Seed germination was 6.7 per cent. Mortality among the 154 seedlings was very heavy. Only 4 plants lived and reached maturity. Three of these were normal triploids and one was a dwarf triploid. An examination of root tips from one of the larger plants showed the triploid number of 21 chromosomes. Triploids are as tall as franciscanas, and the leaves and buds are intermediate in size between the diploid and tetraploid. The pollen of triploids is about 40 per cent good, one half of the grains being 3-lobed and one half 4-lobed. This proportion of 3- and 4-lobed pollen grains is characteristic of the triploid (Davis 1933, pp. 298-299), and a distinguishing character in contrast to the pollen conditions in franciscana and in the tetraploid.

Culture 35.54, table 1, from the cross franciscana x tetraploid with its germination of only 1.6 per cent was even more highly seed sterile. Of the 10 seedlings 7 survived to give 6 normal triploids and one dwarf triploid. The rosette of one of the normal triploids is shown in text figure 1, C, and

a chromosome set from the root tip, 21 chromosomes, is given in text figure 2, C. The seven plants had the characters of triploids including the 3- and 4-lobed pollen grains.

Culture 35.55, tetraploid x franciscana, was the reciprocal of culture 35.54. The very low germination of 2.8 per cent gave 44 seedlings of which only 8 survived. From one of the young rosettes, text figure 1, D, the triploid chromosome count of 21 was obtained, as shown in text figure 2, D. The seven plants which reached maturity had pollen 35-40 per cent good with 3- and 4-lobed grains in about equal proportions.

Progenies from Triploids

There is shown in table 2 progenies from three triploid plants. Culture 35.42 was from a triploid out of a cross franciscana x tetraploid. The rather low germination of 19.4 per cent gave 72 seedlings of which 61 died young leaving only 11 plants to be set in the field. These developed into 4 franciscana-like plants, 2 tetraploid

Table 1

Reciprocal crosses between Oenothera franciscana and the tetraploid.

Culture	Parents	Percent of Germination	Seedlings	Died	Plants	Good Pollen
35.53	Tetraploid x franciscana 33.51-78 x 33.22-385	6.7	154	150	3 normal triploids 1 dwarf triploid	40%: half 3 lobed, half 4 lobed
35.54	Franciscana x tetraploid 33.61-21 x 33.51-21	1.6	10	3	6 normal triploids 1 dwarf triploid	40%: half 3 lobed, half 4 lobed
35.55	Tetraploid x franciscana 33.51-21 x 33.61-21	2.8	44	36	7 normal triploids 1 triploid-like rosette	40%: half 3 lobed, half 4 lobed

Table 2

Progenies from triploid plants out of crosses between *Oenothera franciscana* and the tetraploid.

Culture	Parent	Percent of Germination	Seedlings	Died	Plants	Good Pollen
35.42	Triploid 33.52-50	19.4	72	61	4 franciscana-like 2 tetraploid rosettes 1 bushy dwarf 4 dwarfs	85-98%, 3 lobed 35%: 4/5, 3 lobed, 1/5, 4 lobed 35-50%: 4/5, 3 lobed, 1/5, 4 lobed.
35.43	Triploid 33.53-4	12.1	44	33	4 franciscana-like 1 triploid 1 bushy dwarf 1 dwarf 4 thick leaved rosettes	98%, 3 lobed 40%; 1/2, 3 lobed, 1/2, 4 lobed 50%; mostly 3 lobed, some 4 lobed 35%; 4/5, 3 lobed, 1/5, 4 lobed
35.44	Triploid 33.53-8	17.5	95	64	12 franciscana-like 2 tetraploids 6 bushy dwarfs 3 dwarfs 2 broad thick leaved rosettes 6 narrow thick leaved rosettes	98%, 3 lobed 20%, 4 or more lobed 40-60%, mostly 3 lobed some 4 lobed. 35%, 3 lobed

rosettes, 1 bushy dwarf and 4 dwarfs. Four young rosettes were photographed from this culture, text figure 3. They included A, franciscana; B, a triploid; C and D, tetraploids; as established by their chromosome sets shown in text figure 4, A, B, C and D. In this culture there were also some types which did not appear to be either diploid, tetraploid or triploid. The chromosome counts from their root tips showed four of them to have 15 chromosomes, text figure 4, E, F, G, H, and one 22 chromosomes, text figure 4, I; the latter died while a rosette. Only one of the four with 15 chromosomes survived, becoming a dwarf. The chromosome count in each of the odd types was one more than the normal number of 14 or 21 chromosomes, and they were consequently hyperploids, most of which were unable to live.

Culture 35.43, table 2, was from a triploid plant out of the reciprocal cross tetraploid x franciscana. With a germination of only 12.1 per cent, the triploid gave 44 seedlings most of which died early; only 11 plants survived in the field. They were 4 franciscana-like, 1 triploid, 1 bushy dwarf,

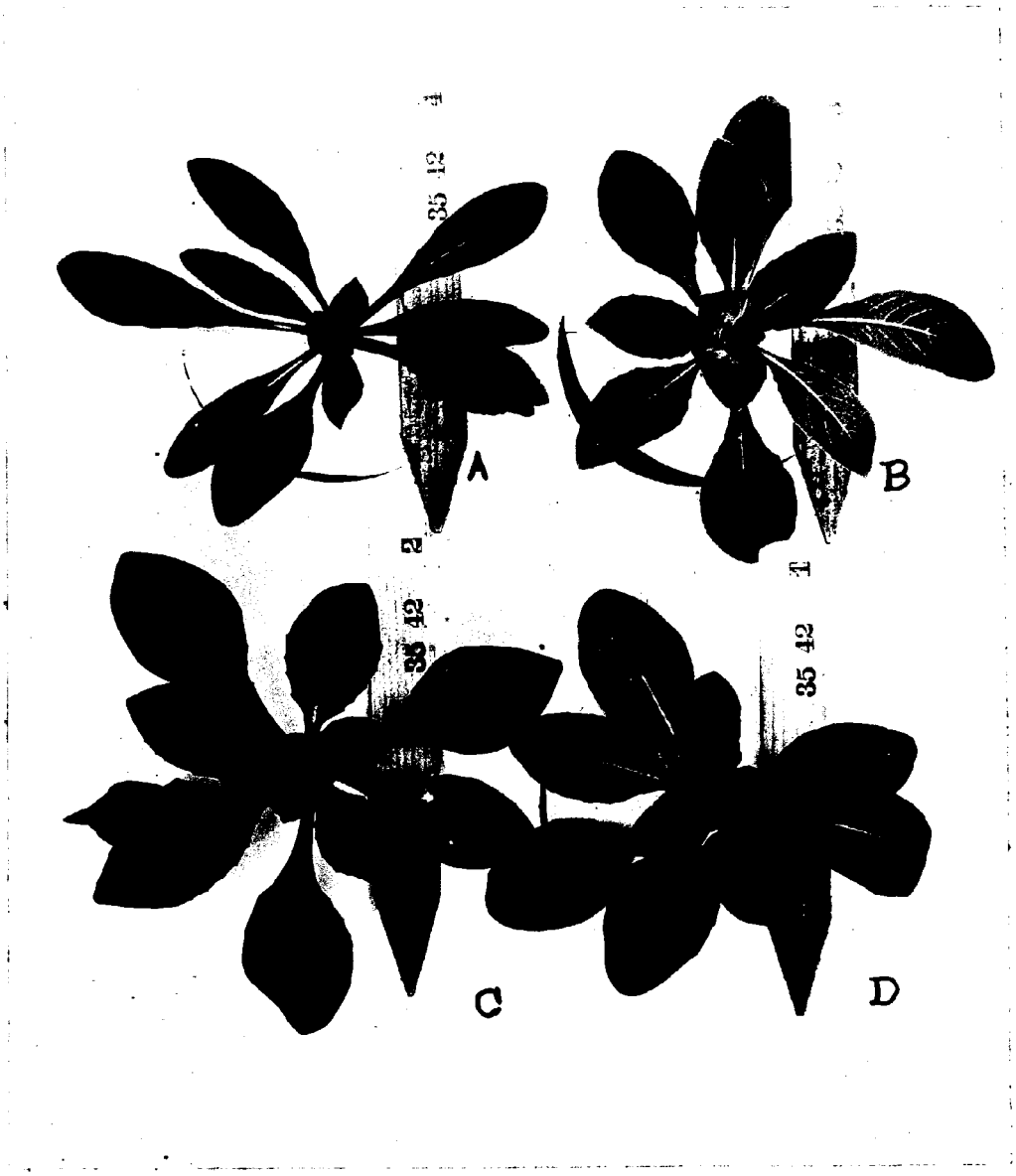
1 other dwarf and 4 thick leaved rosettes. A group of four young rosettes selected from the above is shown in text figure 5; A and B, franciscana-like with 14 chromosomes; C, a triploid with 21 chromosomes; and D, a tetraploid with 28 chromosomes. Chromosome groups from these four plants are given in text figure 6, A, B, C, D, and counts from two other plants established one with 14 chromosomes, E, which became a dwarf, while the other, a hyperploid, F, with 16 chromosomes, died in the field.

Culture 35.44, table 2, was from another triploid plant out of the cross tetraploid x franciscana. A seed germination of 17.5 per cent gave 95 seedlings of which 31 survived, becoming 12 franciscana-like plants, 2 tetraploids, 6 bushy dwarfs one of which examined had 15 chromosomes, 3 other dwarfs, 2 broad thick-leaved rosettes and 6 narrow thick-leaved rosettes. There is shown in text figure 7 four types of young rosettes. The examination of their root tips established 14 chromosomes in plants A and C, 15 chromosomes in B, and 28 chromosomes in D, text figure 8. Plant A died

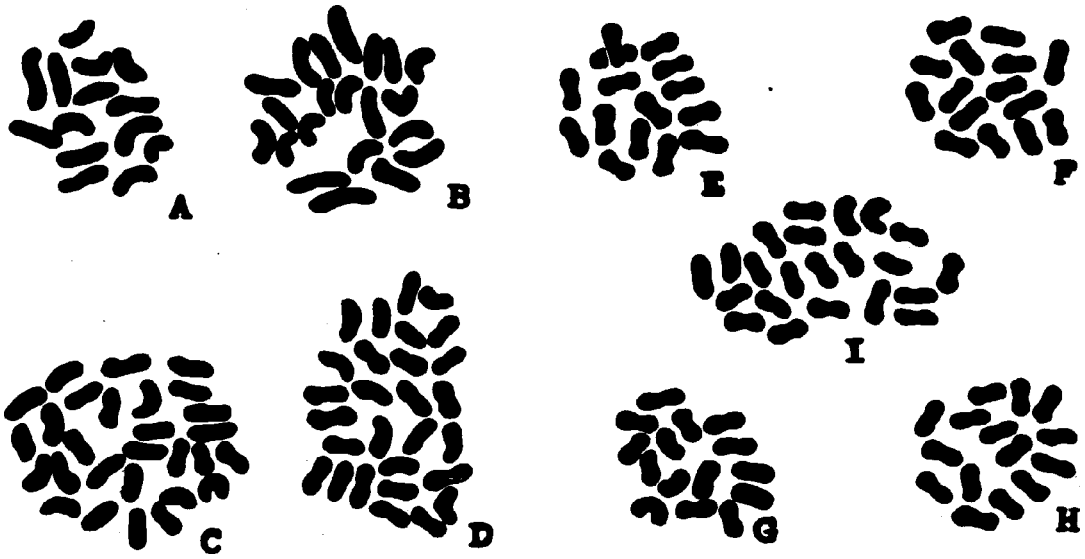
in the field, plant B with 15 chromosomes became the bushy dwarf described and figured by Anderson (1933), plant C grew to be a vigorous franciscana and plant D became a tetraploid.

The breeding behavior of the triploid plants as described above presented the following peculiarities. The progenies consisted of varied types of plants. Of these there were 20 franciscana-like, 4 tetraploids, 1 triploid, 8 bushy dwarfs, 8 other dwarfs and 12 remaining as large rosettes. The examination of the root tips of the representatives with irregular chromosome counts established 6 of them with 15 chromosomes, 1 with 16 chromosomes and 1 with 22 chromosomes, all of them being hyperploids. As will be described later, meiosis in the triploid presents irregularities in the chromosome segregation, giving to the resulting nuclei different numbers of chromosomes ranging from 7 to 14. The combination of the gametes that carry 7 chromosomes usually produce franciscana-like plants, but there are occasional dwarfs with 14 chromosomes. Evidently 7 chromosome gametes more frequently unite to form viable seeds than

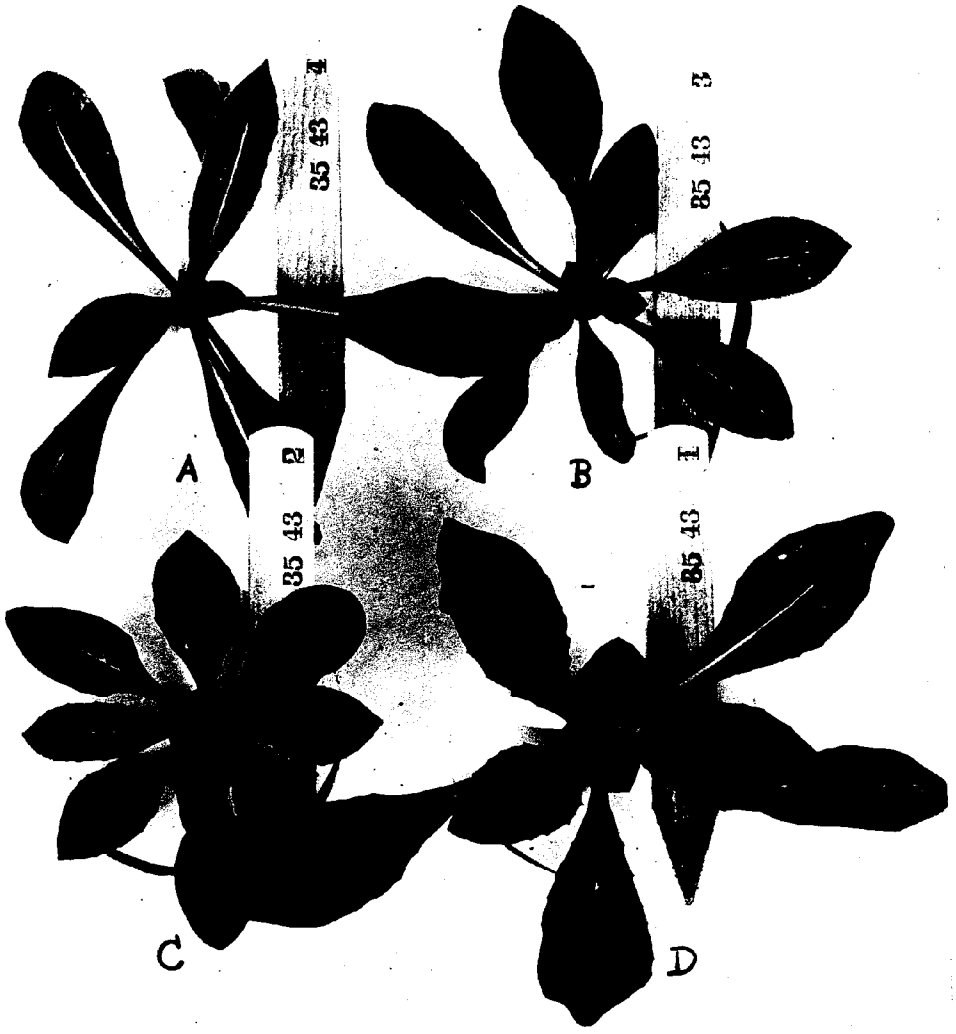
any other chromosome combinations. The union of two 14 chromosome gametes naturally gives the tetraploid. When a gamete of 7 chromosomes unites with a gamete of 14 chromosomes, a triploid results. The 15 chromosome types probably come from the association 7 + 8 and the 16 chromosome variant from the fusion of two 8 chromosome gametes or from a combination 7 + 9. The union of gametes with 14 and 8 chromosomes respectively may be responsible for the 22 chromosome variant. The total number of seeds from which these cultures were derived was 1172. Of these the large number of 961 proved to be sterile. The present cytological studies as well as those of Davis showed the chromosomes to be distributed in meiosis in numbers ranging from 7 to 14 with the probability of even a greater range. By chance matings zygotes might be expected with chromosome counts of from 14 or less to 28 or perhaps somewhat more. Since no other combination than those of 14, 15, 16, 21, 22 and 28 have as yet been found, it seems propable that other combinations are not likely to give viable zygotes or embryos.



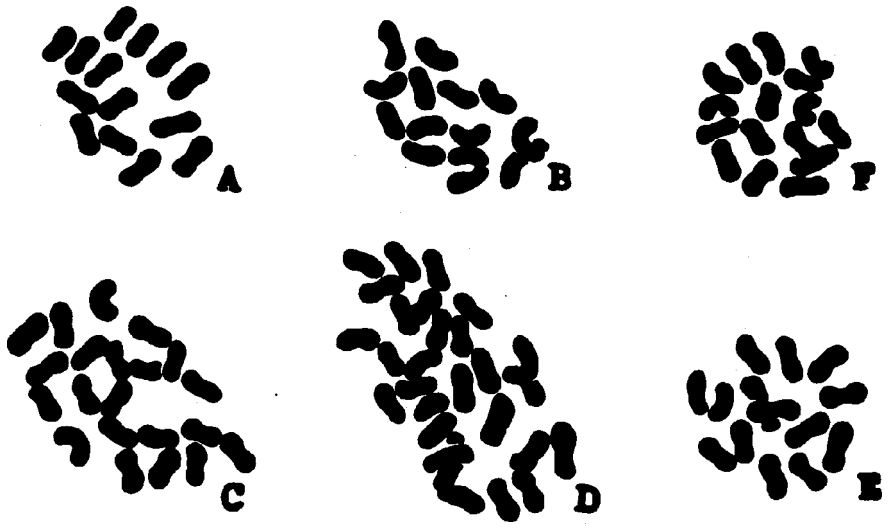
Text figure 3. Four young rosettes, segregates in the progeny from a triploid plant out of a cross franciscana x tetraploid. A, diploid, franciscana-like. B, triploid. C and D, tetraploids.



Text figure 4. Chromosome counts from the root tips of the four rosettes (A, B, C, and D) shown in text figure 3, and also from five other types of rosettes in the same culture. A, diploid, franciscana-like, 14 chromosomes. B, triploid, 21 chromosomes. C and D, tetraploids, 28 chromosomes. E, F, G, and H, hyperdiploids each with 15 chromosomes. I, hypertriploid with 22 chromosomes.



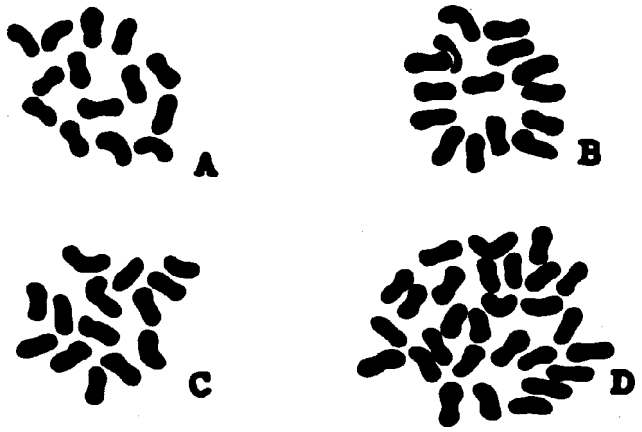
Text figure 5. Four young rosettes, segregates in the progeny from a triploid plant out of the cross tetraploid x franciscana. A and B, diploids, franciscana-like. C, triploid. D, tetraploid.



Text figure 6. Chromosome counts from the root tips of the four rosettes (A, B, C and D) shown in text figure 5 and also from two other types of rosettes in the same culture. A and B, diploids, franciscana-like, 14 chromosomes. C, triploid, 21 chromosomes. D, tetraploid, 28 chromosomes. E, a dwarf, 14 chromosomes. F, hyperdiploid, 16 chromosomes.



Text figure 7. Four young rosettes, segregates in the progeny from a triploid plant out of the cross tetraploid x franciscana. A, diploid, franciscana-like. B, hyperdiploid, 15 chromosomes, became bushy dwarf. C, diploid, typical franciscana. D, tetraploid.



Text figure 8. Chromosome counts from the root tips from the four rosettes (A, B, C and D) shown in text figure 7. A, diploid, franciscana-like, 14 chromosomes. B, hyperdiploid, bushy dwarf, 15 chromosomes. C, diploid, typical franciscana, 14 chromosomes. D, tetraploid, 28 chromosomes.

MEIOSIS IN THE MEGASPOROCYTES OF *OENOTHERA FRANCISCANA*, THE TETRAPLOID AND THE TRIPLOID

Oenothera franciscana

The general thickening of the delicate chromatic threads during and following synizesis in the megasporocyte takes place very slowly, and in this process the threads are greatly reduced in length apparently by condensation, plate I, figure 1. Some parts of the threads are much thicker than others, and the thicker portions as a rule lie more or less in the central portion of the mass while the thinner parts are thrown out in loops at the periphery.

The greater the contraction of the threads, the denser becomes the whole chromatic mass. Constrictions then begin to appear in the threads which form loops that interlock with one another, figure 2. As the looped threads loosen it becomes evident that sections between some of the constrictions are chromosomes. Finally in diakinesis all the chromosomes become definitely outlined and grouped as 7 pairs, figures 3 and 4. In later diakinesis the chromosomes

become smaller because they are more condensed and more sharply outlined. Without exception the chromosomes are in pairs, figure 4. No circles of four were found such as was reported by Cleland (1922) in earlier material of this line of *franciscana*.

At metaphase of the first meiotic division the 7 pairs lie evenly disposed on the spindle, figure 5, and their segregation proceeds with no irregularities. During anaphase a lengthwise division appears in each chromosome so that 7 split chromosomes pass to each pole of the spindle, figure 6, and enter the daughter nuclei, figure 7.

The nuclei grow larger during interkinesis and the halves of the chromosomes separate to give characteristic X-shaped pairs, figure 7. The megasporocyte divides. The chromosomes become much condensed during prophase and appear as very small bodies on the spindle at metaphase of the second meiotic division, figure 8. The count of 7 chromosomes is easily made in late anaphase, figure 9, and early telophase, figure 10. The nuclei increase

rapidly in size, and the chromosomes lengthen and become connected by delicate threads to form a loose net. There results the tetrad of four megaspores in a line.

The Tetraploid

The nucleus of the tetraploid appears to come out of synizesis through a gradual loosening and expansion of the chromatic threads which have been passing through a period of condensation. As the threads thicken and contract during pachynema they twist and coil in loops, plate II, figure 11. Connections may be noted between closely associated threads which are frequently not evenly condensed. Thinner threads may form loops in the peripheral part of the mass, while thicker threads usually occupy the central portion. As the contraction continues the threads become more evenly outlined and more conspicuously coiled and twisted around one another. The whole mass gathers at one side of the nucleus giving the stage called "second contraction." At this time constrictions begin to make their appearance along the threads.

Lengthwise splitting of the threads may sometimes be recognized even at this early stage, and at the same time the individual chromosomes become outlined by the further constrictions, figure 12. Soon the mass begins to loosen through a separation of the chromosomes. The beginning is indicated in figure 12 and later stages are illustrated in figures 13 and 14, which show a splitting of the chromosomes and delicate double thread connections between them.

The nucleus enters diakinesis, figures 13 and 14, when the chromosomes separate out from the mass in pairs, in fours and as single chromosomes. During this history the chromosomes are subjected to still further condensation, and therefore they appear much more sharply outlined. They may become quite evenly distributed throughout the whole nuclear cavity. As indicated above the chromosomes are found in groups of three main types: quadrivalents, bivalents and univalents, as illustrated in figure 14. The quadrivalents are either in circles or in chains. The bivalents are either joined end-to-end or in rings with both ends attached. Most of the univalents lie free, but some of them are linked to bivalents. These types

of configuration were found to be very common by Seitz (1935) in his studies on the microsporocytes of a tetraploid, franciscana gigas. The comparatively small number of trivalents and the chains of six and eight which he observed were not noted in the megasporocytes of the present investigation.

The large number of the chromosomes makes it difficult to follow their arrangement on the equatorial plate of the spindle in the first meiotic division, figure 15. A general pairing at metaphase is usually evident, giving numerous bivalents, but univalents are frequently present. It would be difficult to recognize quadrivalents in the crowded assemblage of chromosomes at metaphase. A lengthwise split in each chromosome is clear in favorable material.

Following the first meiotic division the nuclei of interkinesis show the chromosomes much more conspicuously split than at earlier stage, figure 16. Most of the nuclei of interkinesis contain 14 split chromosomes, but varying numbers below 14 are found

in many of the nuclei. This is due to the lagging of the chromosomes on the spindle of the first division and their consequent failure to be incorporated in the nuclei of interkinesis.

Two daughter cells result from the first division, and shortly the nuclei of interkinesis pass simultaneously into the second meiotic division, figure 17. The split chromosomes form a very well definedequatorial plate and each half generally passes to an opposite pole. In this division the lagging of the chromosomes on the spindle is also found, giving further irregularities in chromosome distribution, figure 18. Following the second division the chromosomes begin to lengthen and the nuclei grow larger. Gradually the chromosomes become connected by fine threads, and there is formed the chromatic reticulum characteristic of the resting nucleus. Cell divisions give the line of megaspores.

The nucleus in the megaspore nearest to the micropyle frequently passes into the resting condition in advance of the other three. In this behavior the tetraploid apparently differs from the material described by Ishikawa (1918) in which nuclei of the

micropylar and chalazal megaspores enter the resting condition while the two intervening ones remain in telophase. As development proceeds there is very evident competition among the four megaspores. As far as observed, with the exception of the cases in which all the four megaspores degenerate, the embryo sac usually develops from the megaspore at the micropylar end.

A striking feature of meiosis in the megasporocyte of the tetraploid is the very early appearance of the lengthwise division of the chromosomes. This division is frequently apparent in late prophase just before diakinesis, and becomes conspicuous in diakinesis. The split appears earlier in the tetraploid than in the diploid and triploid.

The Triploid

In much the same manner as in the diploid and tetraploid the pachynema of the triploid emerges from synizesis with characteristic loops. An open spireme stage is brought to close by the thickening and shortening of the threads, plate III, figure 19,

causing them to twist about one another so closely that they finally appear like a knot, the stage of "second contraction," figure 20. During this stage constrictions appear and finally individual chromosomes become evident. Gradually the knot loosens and the chromosomes come out either singly or attached to one another in groups of two or three, figures 21, 22. Pairs of threads connecting the ends of chromosomes are sometimes clear, together with further evidence of a lengthwise division of each chromosome.

This brings the history to the stage of diakinesis, figure 22. At this time the chromosomes have become sharply outlined and appear as univalents, bivalents and trivalents. The bivalents are pairs either end to end or with both ends joined to form a ring. The trivalents are usually in chains. The chromosomes in this stage are more or less evenly distributed throughout the nucleus. These types of chromosome configuration were reported by Davis (1933) in the microsporocyte of this triploid. Catcheside (1931) reports for a triploid from Oenothera pycnocarpa larger groups of 4, 5 and 6

chromosomes, but such have not appeared in this material.

A metaphase of the first meiotic division is shown in figure 23 with the following groups of chromosomes: four trivalents, three bivalents and three univalents, a complete set of 21 chromosomes. The arrangement of the chromosomes on the equatorial plate seems to follow no system: some trivalents present the middle chromosome toward one pole, and some present the middle chromosome toward the other pole. Consequently segregation will inevitably be irregular. This was also true in the microsporocytes of the same triploid described by Davis (1933) who noted some cells with 7 trivalents. Such complete sets were not found in the megasporocytes of this material. Although there is indication of a lengthwise split in these metaphase chromosomes, it is not so clear as that in the tetraploid.

The chromosomes are apparently distributed at random by the first division resulting in counts of 7-14, 8-13, 9-12 and 10-11 in the nuclei of telophase and interkinesis. The lagging of chromosomes, as shown in figure 24, is of high frequency greatly increasing

the irregularities of chromosome segregation. Figure 25 shows 5 lagging chromosomes left between daughter nuclei which have 9 and 7 chromosomes respectively. The lengthwise fission of the chromosomes becomes conspicuous at anaphase to give split chromosomes to the nuclei of interkinesis.

The stage of interkinesis is followed shortly by the spindles of the second division on which the split chromosomes take the usual orderly position, figure 26. In figure 26 there is also shown a small spindle with two split chromosomes, evidently lagging chromosomes such as are present in figure 25. During the second division the halves of each split chromosome pass to the opposite poles except for occasional irregularities of lagging, figure 27.

Chromosomes outside of the spindles of the second division may develop one or two small nuclei in addition to the four that make up the tetrad, figure 28. Since there is always strong competition among the normal megaspores and only one of the four survives, such extra nuclei are certain to break down.

During telophase of the second division the

chromosomes elongate and finally become connected by threads when their outlines are largely lost, figure 28. The nuclei now enter the resting condition. Usually the cell at the micropylar end of the tetrad rapidly enlarges and develops into the embryo sac.

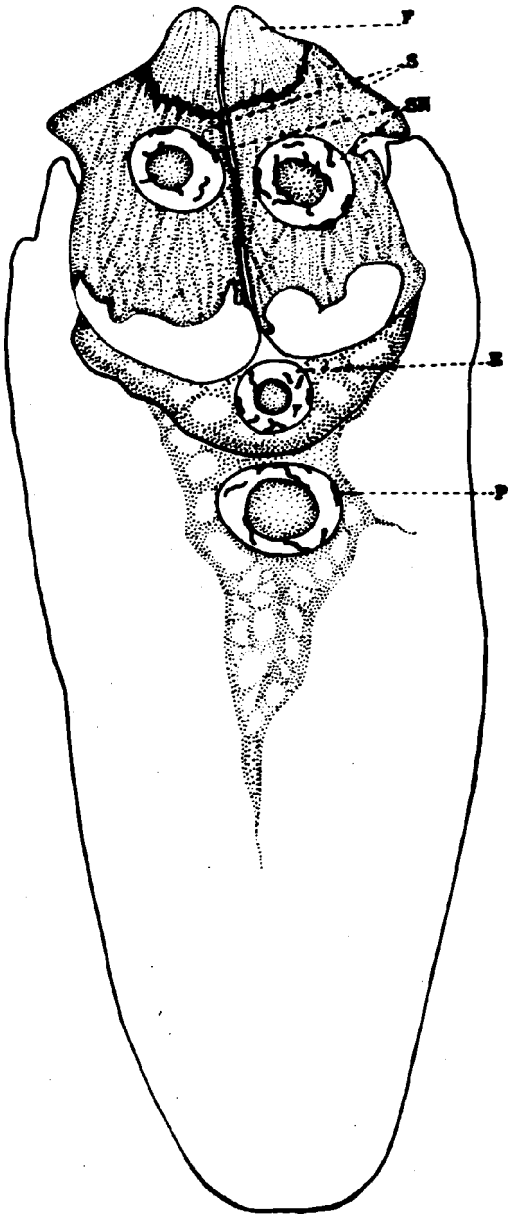
During the period of competition among the four megaspores, some ovules were found in which distintegration of all four cells had taken place. Perhaps the chromosome distribution among those cells may be so irregular at times that none of them recieves a chromosome group that is able to carry forward the development of a female gametophyte.

THE FEMALE GAMETOPHYTE OF THE TETRAPLOID

Because of the large size of the embryo sac in the tetraploid, a study was made of its structure, text figure 9. The two synergids, S, taper at the apex into well developed filiform apparatus, F, described by Ishikawa (1918). In the lower portion of each synergid there is a large vacuole. Between the filiform apparatus and the vacuole lies the synergid nucleus, SN. The egg cell is just below the two synergids. A single polar nucleus in most cases lies directly under the egg. The polar nucleus, N, is much larger than the egg nucleus, E. The structure of this four nucleate embryo sac thus agrees with the description of Ishikawa (1918). The gametophytes of the diploid and the triploid are smaller in size but similar in structure.

Twin embryo sacs were occasionally observed in the material of the tetraploid, triploid and diploid. They usually lie one above the other and are probably derived from different megaspores, although twin megasporocytes were often observed. As a rule the upper one of the megaspores in a tetrad is much far-

ther along in its development and more likely to function. Twin embryo sacs have been reported in several earlier studies on Oenothera.



Text figure 9. Female gametophyte of the tetraploid. F, filiform apparatus. S, synergid. SN, synergid nucleus. E, egg nucleus. P, polar nucleus.

SEED STERILITY IN THE RECIPROCAL CROSSES.

Both Oenothera franciscana and the tetraploid have been described with respect to their genetics and the events of meiosis. The diploid, franciscana, is homozygous with 7 pairs of chromosomes and is highly stable with seed fertility of about 90 per cent. The tetraploid has also proved to be very stable through seven generations, with an average seed germination of about 60 per cent. Except for two franciscana-like plants, probably from egg developing parthenogenetically, there have been only occasional dwarfs. The chromosome segregation of franciscana is regular with production of 7 chromosome gametes, and that of the tetraploid is sufficiently regular to give large numbers of female gametophytes with 14 chromosomes as proved by its pure breeding.

Because of the high fertility of these two plants, franciscana and tetraploid, it might be expected that the seed fertility of the reciprocal crosses would also be good. The facts however showed surprisingly high degrees of seed sterility. The average seed fertility from six cultures in the cross franciscana x

tetraploid was 13.5 per cent and that of the reciprocal was 11.9 per cent. Since the parent plants were free from suspicion of carrying lethal genes, this seed sterility might result from more simple factors on which evidence could be obtained through cytological and embryological studies. Such studies have been made and the results are presented below.

In charts 1 and 2 an attempt is made to show graphically for the parent plants and their hybrids the rate of pollen tube growth, time of appearance of endosperm and embryo development, the disintegration of certain zygotes and very numerous embryos, the proportion of shriveled ovules, and finally the percentage of good and bad seeds. The conclusions shown graphically in chart 1 are derived from the data presented in table 3.

Through the study of large numbers of ovules, table 3, it became clear that at least three factors had played their parts in bringing about the high degrees of seed sterility in the reciprocal crosses; namely, (1) pollen tube growth, (2) fertilization and endosperm growth, and (3) embryo development. The material in this comparative study will therefore be grouped under these three headings.

Table 3

Conditions in ovules studied at different periods after pollination.

Plants	Hours after pollination	Ovules examined	Sacs reached by pollen tubes	Endosperm growth	Zygote division	Zygote disintegration	Embryo development
Franciscana	48	375	334				
	72	524		513	11		
	96	400			392		
	120	486					477
	144	414					406
Tetraploid	48	361	155				
	72	433	390	193			
	96	501	451	311	85		
	120	384		331	234		
	144	348			49	19	244
Tetraploid x franciscana	48	496	110				
	72	374	199	19			
	96	568	427	56			
	120	471	354	259	43		
	144	369		240	196	34	
Franciscana x tetraploid	48	457	10				
	72	502	216	11			
	96	434	287	27			
	120	396	278	119	28		
	144	476		270	237	39	

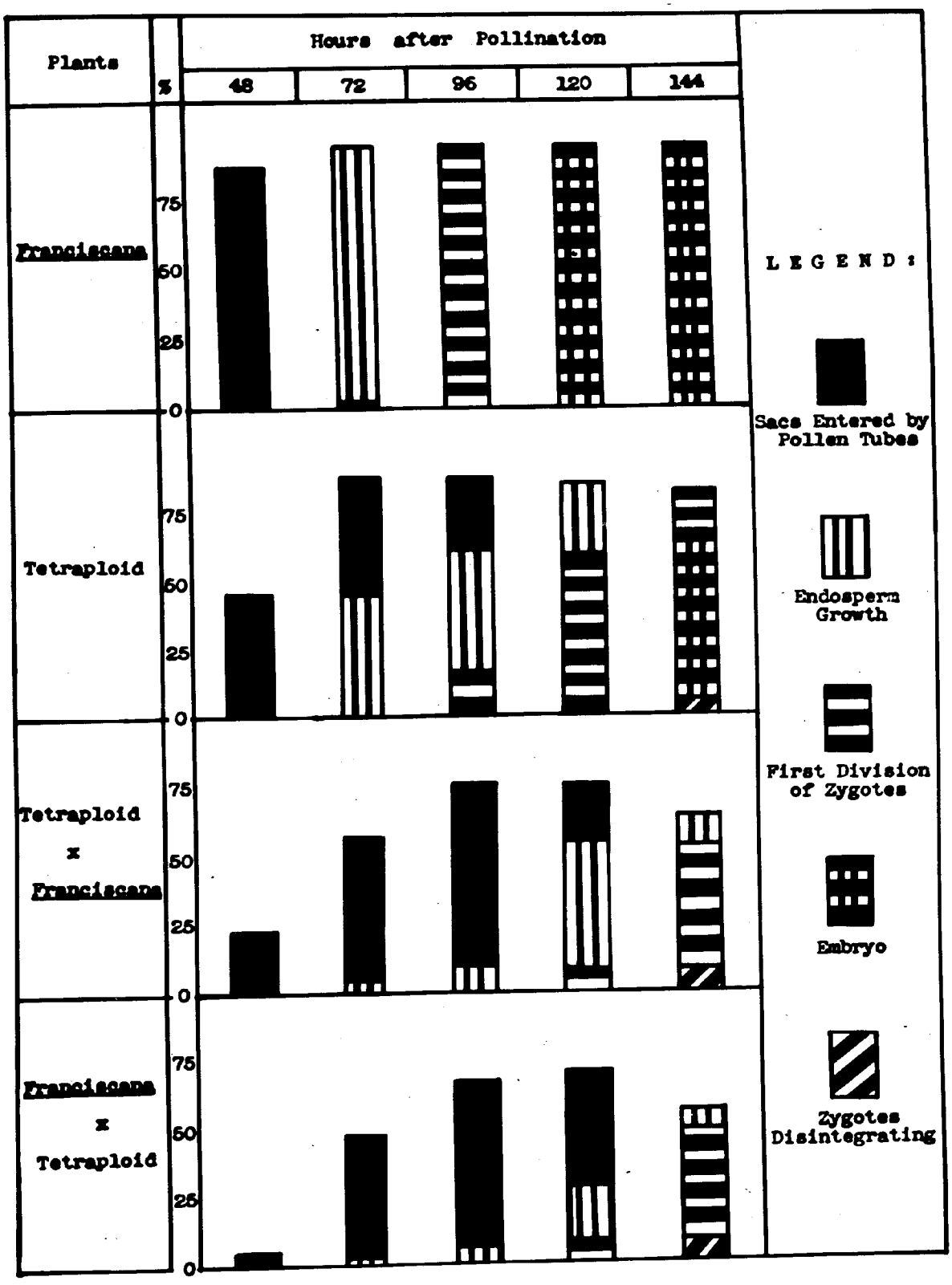

Plants
%
Hours after Pollination
48
72
96
120
144
Franciscana
Tetraploid
Tetraploid x Franciscana
Franciscana x Tetraploid
75
50
25
0
LEGEND:
Sacs Entered by Pollen Tubes
Endosperm Growth
First Division of Zygotes
Embryo
Zygotes Disintegrating

Chart 1.

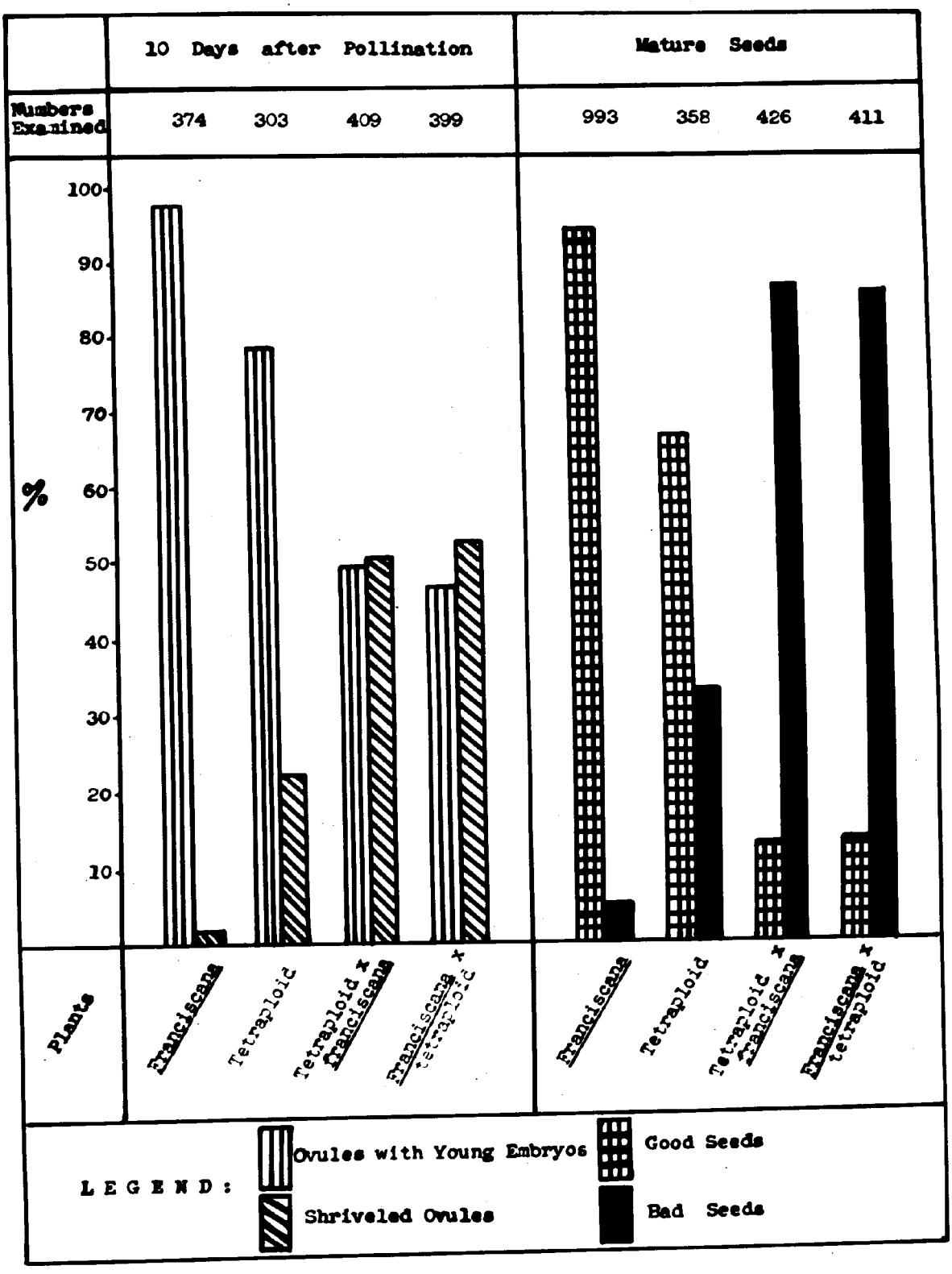

10 Days after Pollination
Mature Seeds
Numbers Examined
374
303
409
399
993
358
426
411
100
90
80
70
%
60
50
40
30
20
10
Plants
Franciscana
Tetraploid
Tetraploid x franciscana
Franciscana x tetraploid
Franciscana
Tetraploid
Tetraploid x franciscana
Franciscana x tetraploid
LEGEND:
Ovules with Young Embryos
Shriveled Ovules
Good Seeds
Bad Seeds

Chart 2.

Pollen Tube Growth

As shown in chart 1, franciscana proved to be most uniform in all phases of development. At 48 hours after pollination tubes had reached 89 per cent of the embryo sacs. At 72 hours after pollination 98 per cent of the ovules showed the beginning of endosperm growth and a few zygotes were in their first cell division. Evidently full growth of the pollen tubes was completed not much later than 48 hours after pollination. The number of embryo sacs studied for each of the above stages is given in table 3.

The pollen tubes of the tetraploid as shown in chart 1 were much slower in their growth than those of franciscana. Only 43 per cent of the sacs were found entered by the tubes at 48 hours after pollination, and it required 72 hours for pollen tubes to reach 90 per cent of the sacs. There was no increase in pollen tube penetration at 96 hours or later, and apparently 10 per cent of the sacs were not entered.

In the cross tetraploid x franciscana the pollen

tubes of franciscana grew very slowly through the style of the tetraploid. At 48 hours after pollination only 22 per cent of the embryo sacs of the tetraploid had been entered by the pollen tubes of franciscana and at 72 hours only 53 per cent. Pollen tubes continued to enter the sacs and at 96 hours after pollination 75 per cent of the sacs had been reached, after which no more tubes appeared to function with the result that about 25 per cent of the sacs were not entered. This slow growth of the pollen tubes in comparison with those of the franciscana and the tetraploid in their own styles is sharply contrasted in the comparative heights of the black columns in chart 1.

In the reciprocal cross, franciscana x tetraploid, the growth of tetraploid pollen tubes in the style of franciscana was even slower. Only two per cent of the embryo sacs were entered by the pollen tubes of the tetraploid 48 hours after pollination, 43 per cent at the period of 72 hours, 66 per cent at 96 hours, and 70 per cent at 120 hours. About 30 per cent of the sacs of franciscana were not entered by pollen tubes of the tetraploid. These peculiari-

ties in comparison with the behavior of the pollen tubes of franciscana and the tetraploid in their own style, and of franciscana tubes in the style of the tetraploid, are graphically indicated in chart 1.

Fertilization and Endosperm Growth

The entrance of the pollen tubes into the embryo sacs and the events of fertilization are described in detail by Ishikawa (1918) in Oenothera mutans and Oenothera pycnocarpa, and the same history is found in Oenothera franciscana, in the tetraploid, and in the two reciprocal crosses. The pollen tube discharges its content over the egg nucleus and the single polar nucleus. Double fertilization then takes place, text figure 10, A, giving a zygote nucleus and primary endosperm nucleus, each of which usually contains two nucleoli of unequal size, the smaller one coming from the sperm nucleus. After fertilization the zygote nucleus enlarges, text figure 10. B, and prepares for the first division, text figure 10. C, late prophase with 21 chromosomes shown, and D, in metaphase. The mitosis of the primary endosperm

nucleus begins much earlier than that of the zygote. There are usually 16 or more endosperm nuclei present before the zygote begins its first division.

Fertilization in franciscana was highly successful in all of the ovules that were entered by the pollen tubes. The development of the endosperm was usually under way as early as 72 hours after pollination, but the zygote nucleus at this time had rarely divided. However, 96 hours after pollination the first mitosis of the zygote had been completed, chart 1.

Fertilization in the tetraploid was usually successful, though the endosperm and the embryo arose more slowly than those of franciscana. Only about 45 per cent of the ovules showed the endosperm beginning to develop at 72 hours after pollination, and only 62 per cent at the period of 96 hours, with 17 per cent of the zygotes at this time in their first division, chart 1. At 120 hours after pollination endosperm was found in 86 per cent of the embryo sacs, but only 61 per cent of the eggs had passed the first division, chart 1. The development of the endosperm and the embryo was therefore much slower than that in franciscana, but this was probably due to slower

growth of the pollen tubes which entered the sacs a number hours later than those in franciscana. It is noteworthy that the tetraploid is behind franciscana at almost all phases of growth. Seeds germinate more slowly and the mature plants usually come to flower about two weeks later than do those of the franciscana.

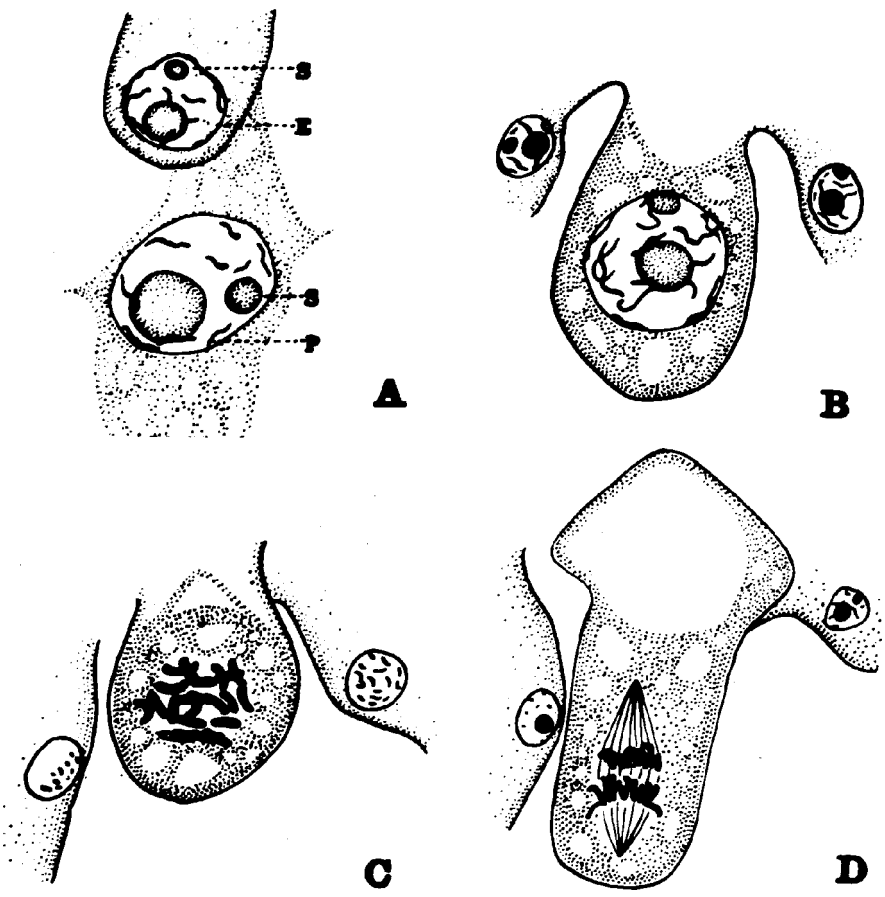
In the two crosses many of the embryo sacs were not entered by the pollen tubes and a number of thosethat were entered failed of fertilization. In both cases the eggs degenerated. The breaking down of some of the unfertilized eggs became evident as early as 72 hours after pollination, text figure 11 A, and of others four or five days after pollination, text figure 11 B. The egg nucleus broke down usually before the polar nucleus. Sometimes sperm nuclei disintegrated in which case there was no fertilization and both egg nucleus, text figure 11 C, and polar nucleus broke down.

Since pollen tube growth in these two crosses was very slow, the developments that followed were therefore slower than those of their parents. In the cross tetraploid x franciscana the endosperm scarce-

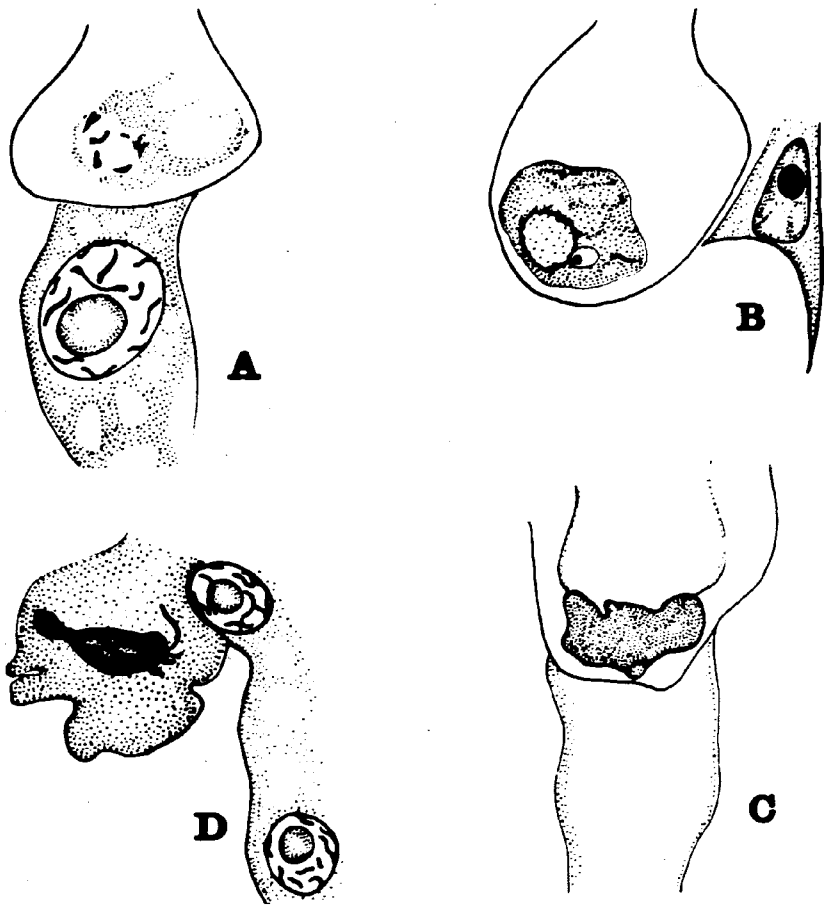
ly began its development before 72 hours after pollination when it appeared in about 5 per cent of the embryo sacs. It was shown in only 10 per cent of the ovules 96 hours after pollination and in only 65 per cent at 144 hours, chart 1. The first division of the zygotes did not take place until about 120 hours after pollination and at 144 hours only 53 per cent of the ovules showed divided zygotes, chart 1. During this period there was evidence of nuclear disintegration in about 9 per cent of the zygotes although endosperm nuclei at this time appeared healthy, text figure 11, D.

In the cross franciscana x tetraploid peculiarities similar to those in the reciprocal, tetraploid x franciscana, described above were found with small differences in the amounts of the irregularities, see chart 1. There was an even slower beginning in the development of endosperm found in only 2 per cent of the sacs at 72 hours, 6 per cent at 96 hours, 30 per cent at 120 hours and finally 57 per cent at 144 hours. The first division of the zygote was not observed until 120 after pollination and at 144 hours only about 50 per cent of the ovules presented the

divided zygotes. Again during this period as in the reciprocal cross there were ovules with degenerating zygotes in sacs that contained developing endosperm. This breaking down of the zygotes was noted in about 8 per cent of the ovules.



Text figure 10. Franciscana x tetraploid. A, double fertilization, two sperm nuclei fused respectively with egg (E) and polar nucleus (P) as shown by the introduced small nucleoli (S). B, zygote with two nucleoli, the smaller one from the sperm nucleus. C, zygote nucleus in late prophase, 21 chromosomes. D, zygote nucleus in first division.



Text figure 11. A, B, and C, tetraploid x franciscana; D, franciscana x tetraploid. A, unfertilized egg disintegrating, polar nucleus appearing healthy. B, sperm nucleus lies in the degenerating egg. C, egg disorganized. D, zygote breaking down, endosperm appearing healthy.

Embryo Development

After making allowance in the hybrids for failure of fertilization and for a certain percentage of zygotes that quickly break down, it cannot be assumed that the remaining ovules will develop into viable seeds. Further abnormalities and irregularities to a great degree take place in these two crosses.

The early stages of the embryo development are the same in franciscana, in the tetraploid, and in the reciprocal crosses. The zygote first divides into a suspensor and a proembryo cell, text figure 12. A. The proembryo cell divides twice longitudinally. B, and the four quadrants divides transversely to give octants, C. Periclinal divisions then take place in all the octants, text figure 12. D. The suspensor cell divides transversely.

As indicated in chart 1 (page 43) the beginnings of embryo development in franciscana were healthy in almost all ovules, text figure 13. A. No irregularities were observed until the seeds were full grown when 53 seeds out of 933 were found to

contain abnormal embryos, a proportion very close to the percentage of the seed germination found by Davis.

In the tetraploid, as shown in chart 1, some 90 per cent of the sacs were entered by pollen tubes, but only about 66.8 per cent of the seeds proved to be viable. Evidence of irregularities in the embryo development in the tetraploid became clear 10 days after pollination, chart 2, when about 22 per cent of the ovules appeared shriveled and only 78 per cent of them showed developing embryos, text figure 13. B. Not all of these embryos continued to grow normally, as numbers of them were found in a state of arrested development from this time on, as shown in text figure 14. B, in contrast to the normal, A. An examination of the mature seeds showed that 119 out of 358 were either empty or with abnormal embryos, text figure 14. D, in contrast to the normal, C, thus giving 66.8 per cent good seeds. Data of Davis on seven generations of the tetraploid show a range in germination from 36 to 81 per cent, with an average close to 60 per cent. It seems probable

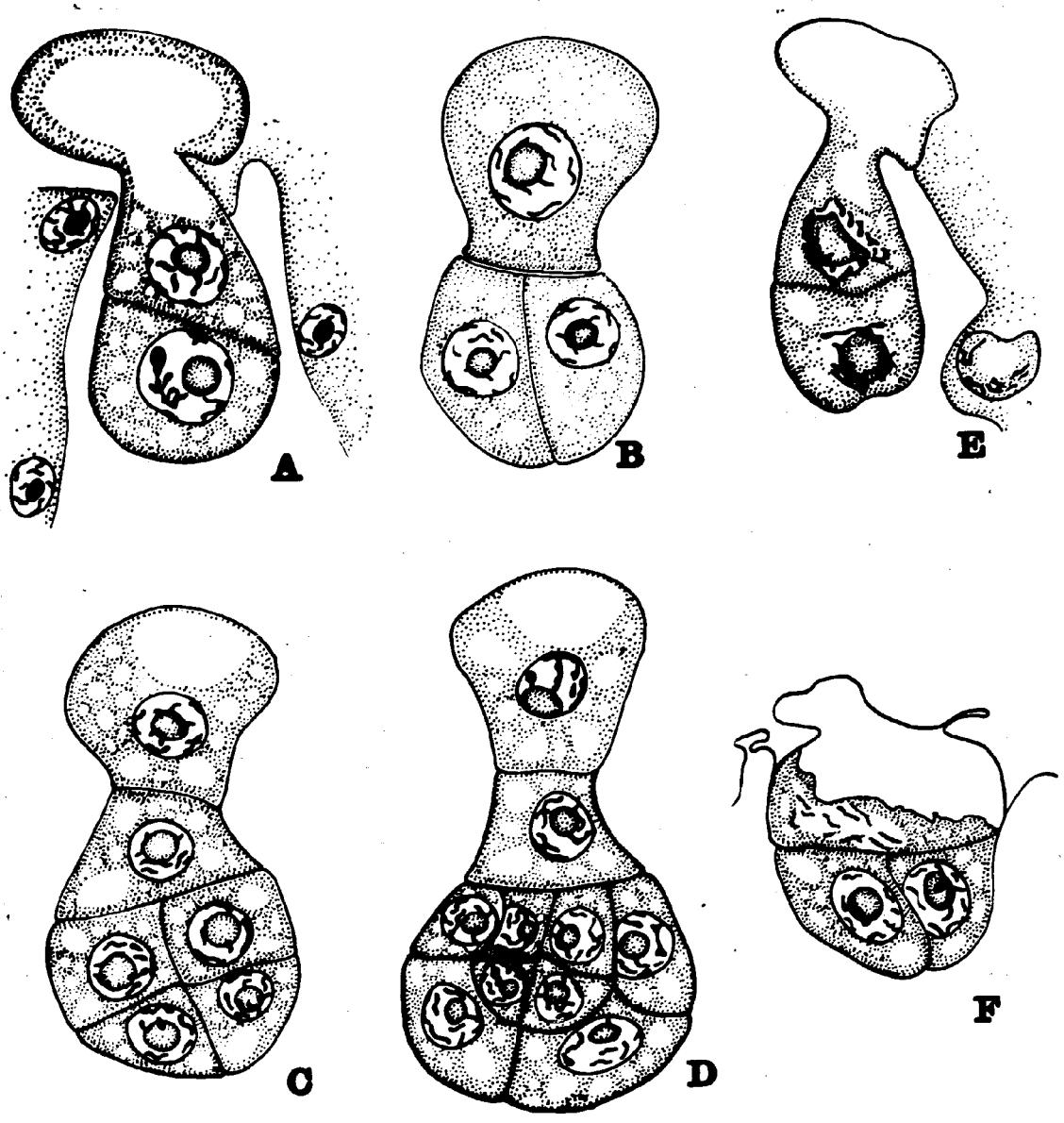
that this sterility may be due to arrested development resulting from irregularities of meiosis which are certain to bring peculiar chromosome combinations into many zygotes. These irregularities have been discussed in the account of meiosis.

The abnormalities of seed development in the two reciprocal crosses stood out in sharp contrast to the regular development shown in their parents. Between 7 and 10 days after pollination, signs of disintegration of the suspensor and the proembryo were frequently observed, text figure 12, E. In some cases the suspensor began to break down after the two celled embryo was formed, text figure 12, F. As has been previously shown, there were in these two hybrids ovules exhibiting a very wide range of intergraded abnormalities: sacs that were not entered by pollen tubes, sacs in which the eggs and the polar nuclei disintegrated with the presence of the pollen tubes, sacs in which zygotes degenerated while endosperm appeared either healthy or otherwise, and cases in which dis-

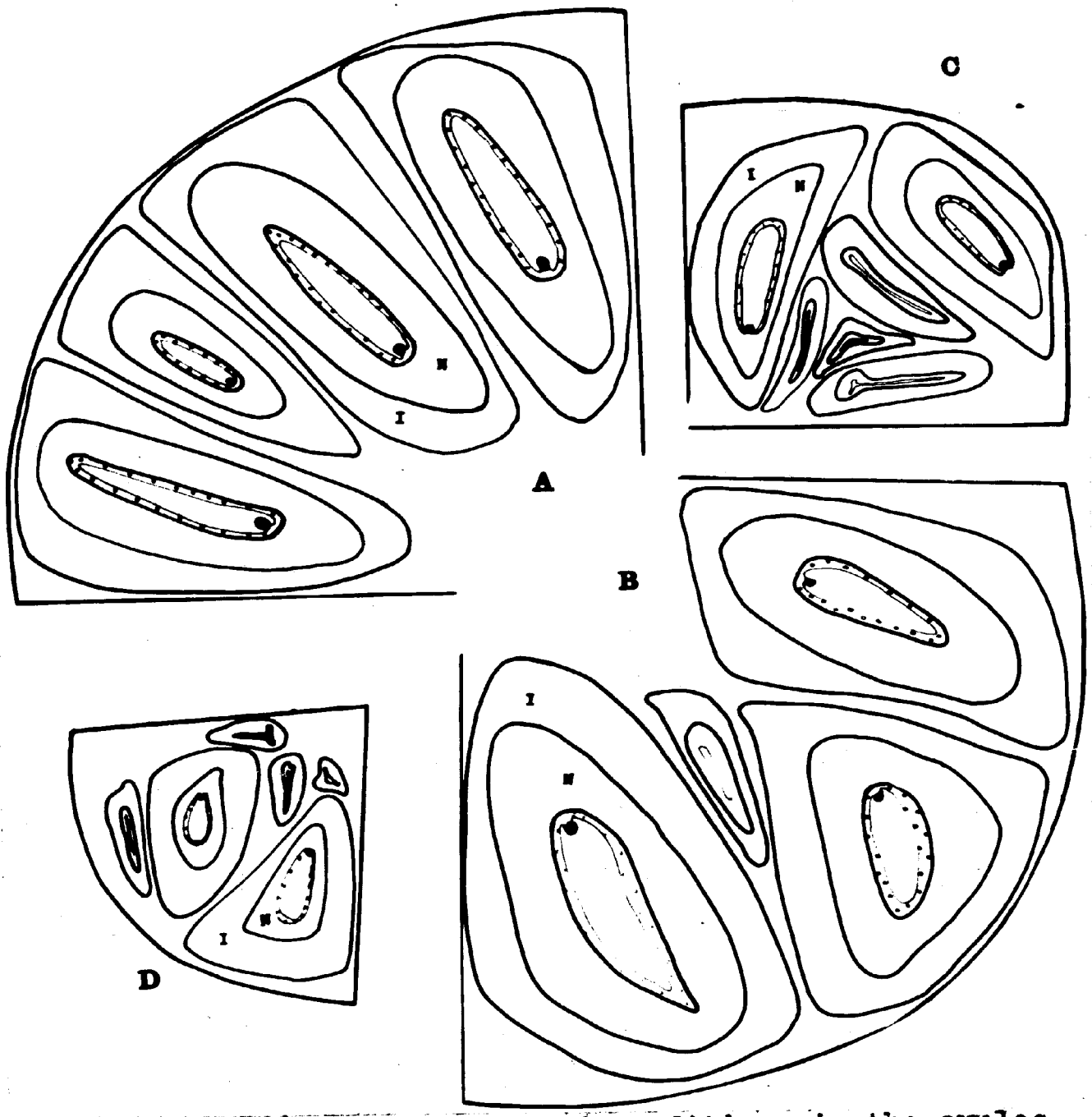
integration of the embryos took place. These irregularities must have been responsible for the shriveled ovules found ten days after pollination as illustrated in text figure 13. C, tetraploid x franciscana, and D, franciscana x tetraploid. These shriveled ovules came to about 51 per cent in the cross tetraploid x franciscana and 53 per cent in the reciprocal cross, chart 2. The embryo development in these two crosses showed the same abnormalities at similar stages. Arrested embryos in many stages may be found. Text figure 15. B shows for franciscana x tetraploid an abnormal embryo 20 days after pollination in comparison with the normal. A, of the same age. Text figure 16. B and C, illustrate degenerated embryos in contrast to the normal, A, in the cross tetraploid x franciscana 25 days after pollination.

In the end the two crosses showed about the same proportion of the bad seeds. In the cross tetraploid x franciscana, out of 426 seed-like structures only 55 were found to have normal embryos, text figure 17 A, normal; B, with abnormal embryos; and C, empty, giving 12.9 per cent good seeds, chart 2. In the

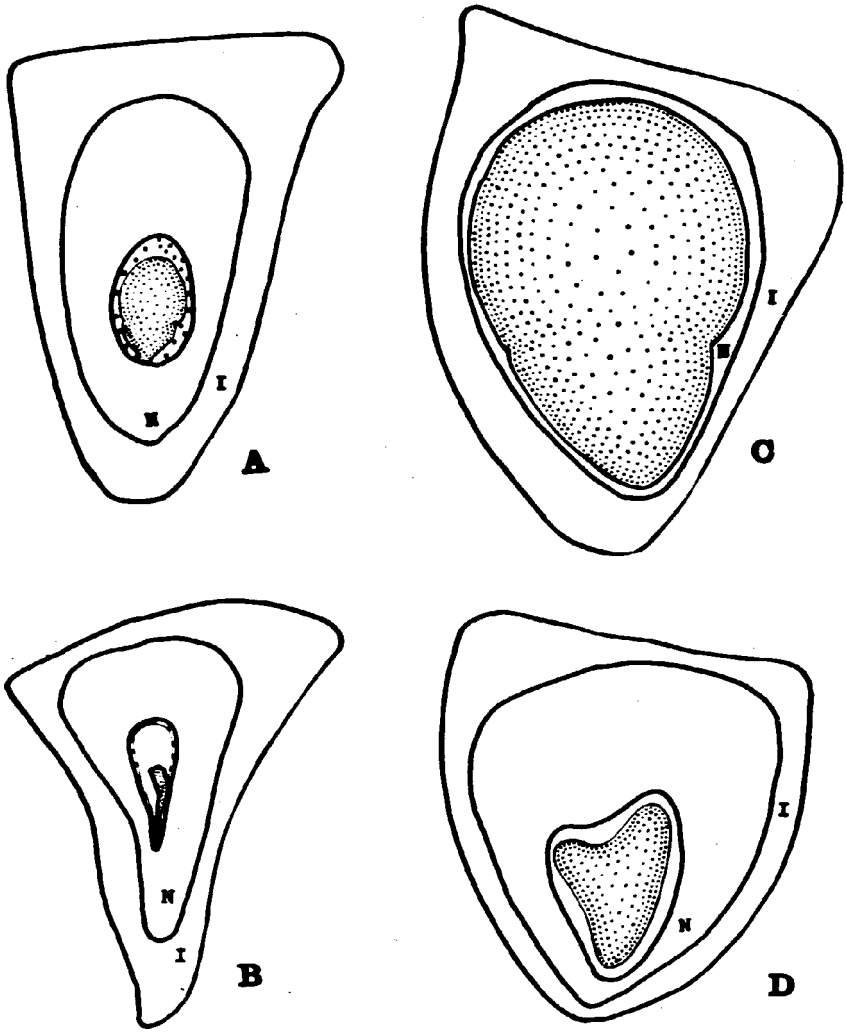
reciprocal cross franciscana x tetraploid, out of 411 mature seeds only 48 had full grown embryos, giving 13.2 per cent good seeds. These low percentages of good seeds are close to the averages of seed germination recorded by Davis, 11.9 per cent for tetraploid x franciscana, and 13.5 per cent for franciscana x tetraploid.



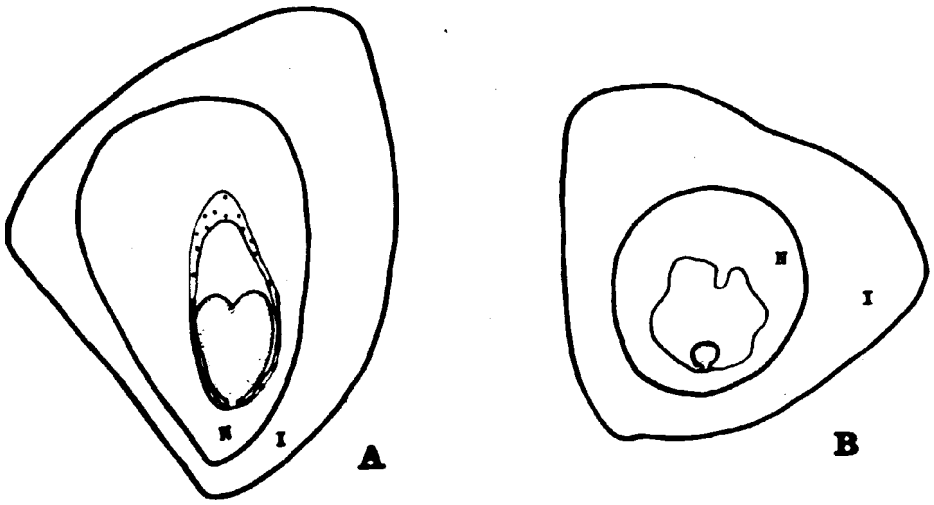
Text figure 12. Franciscana x tetraploid, early stages of embryo development. A, zygote divided to give proembryo and suspensor cells. B, proembryo cell divided lengthwise to give quadrants. C, quadrants divided transversely giving octants, suspensor now 2-celled. D, periclinal division in the octants. E and F, degenerating embryos.



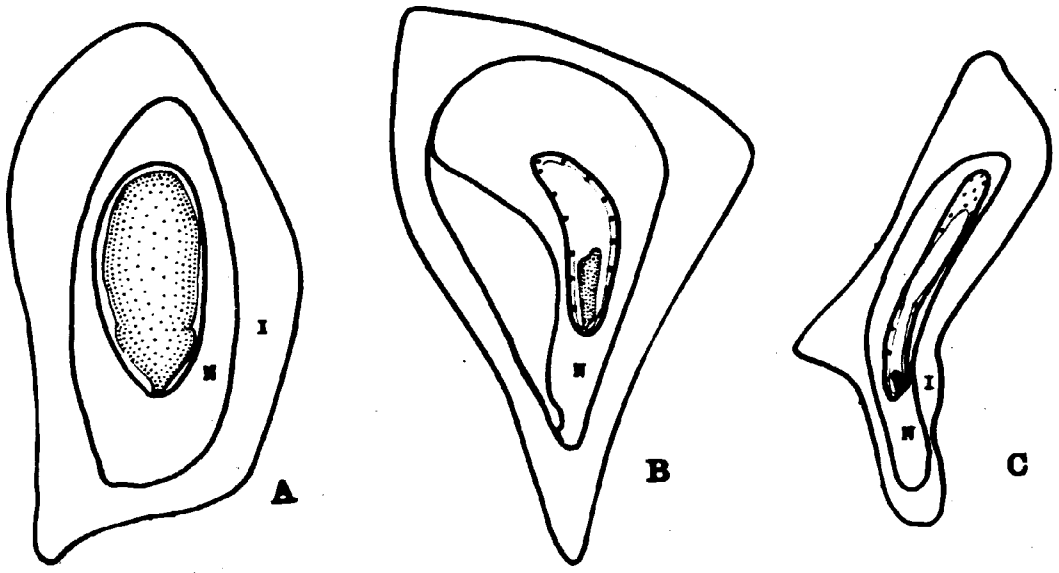
Text figure 13. Comparison of conditions in the ovules from four plants at 10 days after pollination, each figure being one fourth of a cross section of the ovary. A, franciscana, ovules uniform and healthy. B, tetraploid, one empty ovule shown. C, tetraploid x franciscana, and D, franciscana x tetraploid, both showing several shriveled ovules. I, integument; N, nucellus.



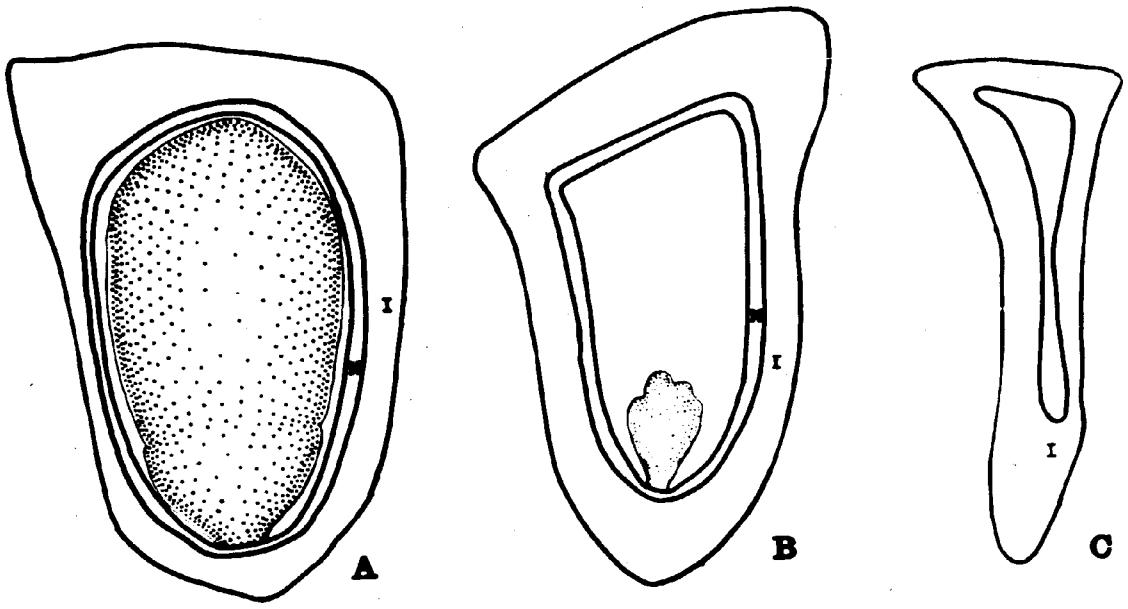
Text figure 14. Tetraploid. A, ovule with healthy embryo 15 days after pollination. B, arrested embryo of the same age. C, mature seed with normal embryo. D, seed with abnormal embryo. I, integument; N, nucellus.



Text figure 15. Franciscana x tetraploid, 20 days after pollination. A, ovule with healthy embryo. B, with abnormal embryo. I, integument; N, nucellus.



Text figure 16. Tetraploid x franciscana, 25 days after pollination. A, normal embryo. B and C, ovules with abnormal embryos. I, integument; N, nucellus.



Text figur 17. Tetraploid x <u>franciscana</u>. A, viable seed with normal embryo. B, with abortive embryo. C, no trace of embryo left. I, integument; N, nucellus.

DISCUSSION

The findings of this investigation are of interest from two standpoints. First, there is the bearing of chromosome segregation in franciscana, in the tetraploid and in the triploid upon their respective breeding behaviors. Second, there is the significance of pollen tube growth in relation to subsequent developments in the ovules of the two crosses, tetraploid x franciscana and franciscana x tetraploid, particularly with respect to their high degree of seed sterility.

Cytological studies on the three forms have been reported by several investigators: for franciscana by Cleland (1922) and Kulkarni (1929), for the tetraploid by Davis (1933) and Seitz (1935), and for the triploid by Davis (1933). Except for the lengthwise splitting of the chromosomes which becomes evident earlier in megasporocytes than in microsporocytes, events of meiosis in the megasporocytes agreed very closely with what has been reported, although no circle of four chromosomes during diakinesis, as observed by Cleland, was found in the

present material of franciscana.

The chromosome segregation in these plants bore a clear relation to their genetical behavior which has been described in detail in the foregoing pages. The more regular the chromosome distribution, the higher was the seed fertility. Chromosome segregation in franciscana proved to be very regular and the seed fertility in this material was 94.7 per cent which comes very close to the average seed germination of about 90 per cent recorded by Davis from 11 generations. The tetraploid had the lower seed fertility of 66.8 per cent which again agrees quite well with Davis' record that gives an average seed germination for 7 generations of about 60 per cent. The irregularities of chromosome segregation in the tetraploid must be responsible for the non-viable zygotes and the abnormal embryos found in about 30 per cent of the ovules, and therefore responsible for the sterile seeds. The very numerous irregularities of chromosome segregation in the triploid correlate well with its breeding behavior, as described on pages 13 to 19, and with its low seed fertility and high seedling mortality.

These peculiarities agree with what has been reported by Capinpin (1933) in a number of triploid Oenotheras. Evidently the varied types of plants were the results of different recombinations of chromosomes, and the sterile seeds must have come as the result of the reshuffling of chromosomes giving incompatible reaction systems.

With irregular gametic chromosome numbers in the tetraploid and especially in the triploid, it was surprising to find that the embryo sacs usually developed from the micropylar megaspores as in franciscana. The disintegration of the whole tetrad was often observed in the ovules of the tetraploid and triploid. Geerts (1909) reported that in Oenothera Lamarckiana the micropylar megaspore was always the one that functioned. He also described ovules in which all four megaspores degenerated. Ishikawa (1918) in his studies of Oenothera nutans, Oenothera pycnocarpa and their hybrids stated that either the micropylar or the chalazal megaspore had an equal chance to function. According to Renner (1921) in Oenothera Hookeri and Oenothera Lamarckiana the micropylar

megaspore is always the functioning one, but in Oenothera muricata either the micropylar or the chalazal megaspore may function due to the fact that only the rigens megaspore is able to develop and the rigens megaspores might be segregated to either the upper or the lower end of the tetrad. Hoeppener and Renner (1929) reported that in Oenothera Lamarckiana gigas the micropylar megaspore developed into the embryo sac while in Oenothera albata (biennis x Hookeri) the embryo sac developed mostly from the micropylar megaspore but sometimes from the chalazal megaspore, and in Oenothera suaveolens either the micropylar or the chalazal megaspore functioned. Taking all these reports into consideration, the micropylar megaspores seem to have a stronger tendency to develop into sacs. The present investigation has shown for the tetraploid and triploid that in most cases, whenever irregularities of chromosome segregation take place, all of the four megaspores receive abnormal chromosome numbers. Judging from the high percentages of the sterile seeds it appears that most of the megaspores must have been able to

develop embryo sacs carrying on their functions until incompatible reactions begin to operate sooner or later in embryo development to arrest its growth. In the cases wherein all four megaspores disintegrate, the cause probably lies in the fact that none of them has received a chromosome set capable of forwarding the development of an embryo sac.

Before discussing the significance of pollen tube growth in relation to the developments in the ovules, a brief review of certain work on Oenothera will be given. Renner (1914) described three types of seeds in Lamarckiana: half of the seeds contained normal embryos, resulting from the union of gametes bearing the velans and gaudens complexes, which gave Lamarckiana again; one quarter of the seeds contained abnormal embryos while the other quarter was empty, the result of the lethal combinations velans-velans and gaudens-gaudens respectively. Thus half of the seeds was non-viable. Since both franciscana and the tetraploid are free from zygote lethals, the high seed sterility of the reciprocal crosses between these two forms must have other causes

than those present in Lamarckiana.

Pollen tube growth in Oenothera has been studied by a number of investigators. Heribert-Nilsson (1911) found that the pollen tubes of Lamarckiana gigas grew more slowly than those of Lamarckiana, each in its own style, just as the gigas plant itself developed more slowly. That the pollen tubes of the diploid grow faster than do those of the tetraploid in their own styles respectively is also true for franciscana and for its tetraploid. Renner (1919) reported for gracilis hybrids derived from the cross Lamarckiana x muricata two types of pollen grains, velans and curvans. In the cross Lamarckiana x gracilis the velans pollen tubes grew more rapidly than the curvans pollen tubes. The velans pollen tubes also grew faster than the gaudens pollen tubes in the cross biennis x Lamarckiana. When the stigma was abundantly pollinated the velutina hybrids appeared much more numerously than the laeta hybrids, but when the stigma was sparsely pollinated, the two hybrids appeared in equal numbers. Heribert-Nilsson (1920 and 1923) reported the rate of pollen tube growth in a Swedish

race of Lamarckiana in which many plants with red midribs were heterozygous Rr, R being the factor for the red midrib and r for the white. He found that the pollen tubes bearing the R factor grew more rapidly than those carrying r. Hiorth (1926) repeated these experiments on a larger scale in the crosses Hookeri x R-Lamarckiana, r-muricata x R-Lamarckiana, R-biennis x Lamarckiana nanella, and R-Lamarckiana x Cockerelli. He adopted the method of contrasting abundant and sparse pollinations and found results very much in accord with those of Renner. The rate of growth was very much faster with the velans pollen than with the gaudens pollen. With respect to the red (R) and the white (r) factors the velans pollen bearing r grew faster than the gaudens pollen but more slowly than the velans pollen bearing R. On the other hand, for the gaudens pollen tubes, those carrying R grew more slowly than those carrying r. The above reports show that there are specific genes affecting the rate of pollen tube growth. Since the pollen tubes of franciscana and of the tetraploid grow well in their own styles,

the tardiness of the pollen tube growth, which is only found in the crosses, must therefore be attributed to some factors in the styles of the female parents.

Buchholz and Blakeslee (1929) reported a series of observations on pollen tube growth in the crosses between the haploid, diploid, triploid and tetraploid plants of Datura. Pollen tubes of the diploid and tetraploid grew well in their own styles there being very few slow growing tubes. Pollen tube growth was also good when the tetraploid female was pollinated from the diploid male, but in the style of the diploid female tetraploid pollen tubes grew poorly.

Müntzing (1930) reported that fertilization occured in the cross Gallopsis Tetrahit (n 16) x Gallopsis pubescens or speciosa (n 8) but not in the reciprocals. Jørgensen (1928) observed a case in the cross Solanum nigrum x Solanum luteum in which the sperm nucleus retained its form in the egg cell for some time, gradually and finally disintegrating. He believed the affinity between the sperm and egg nuclei to have become lessened.

Watkins (1932) from his investigations on wheat concluded that fertilization does not always follow normal growth of pollen tubes and that quantitative relations between gametes as expressed in chromosome content, may prevent fertilization. As to the pollen tube growth, he considers the normal chromosome relation between the tissue of the style and the pollen tube to be 2n : n. If the chromosome count in the style is more than double that of the pollen tube, the growth of the pollen tube is generally normal although sometimes it may be slightly affected, whereas if the chromosome count of the style is less than double that of the pollen tube, its growth is then greatly retarded. In another words the cross of a high chromosome number female to a low chromosome number male is more likely to succeed than the reciprocal.

As has been carefully described and shown in chart 1, the pollen tube growth of franciscana and of the tetraploid was good in their own styles. Growth was poor when the tetraploid was pollinated by franciscana (4n x n) and even poorer in the reciprocal cross, franciscana x tetraploid (2n x 2n).

This seems to agree with what has been reported on Datura and on wheat as discussed above. The relation between tissue of the style and the pollen tube with respect to chromosome number, as proposed by Watkins, seems to offer an explanation for the slow growth of pollen tubes in the crosses, tetraploid x franciscana and franciscana x tetraploid, in which the pollen tube growth was delayed from 24 to 48 hours.

From the discussion so far, the conclusion seems justified that the irregular developments in the ovules of the two crosses are likely to have resulted (1) partly from the irregularities of the chromosome segregation in the tetraploid, but (2) chiefly from the slow growth of the pollen tubes. Slow growth of the pollen tubes will result in the aging of gametes, a factor believed to be important to an understanding of the problem of sterility in the reciprocal crosses between franciscana and the tetraploid.

Since the irregularities of chromosome segregation in the tetraploid are held to be responsible for only a limited amount of non-viable zygotes and abnormal embryos as found in the tetraploid, the slow growing tubes are considered to be the main cause of

the following events. First, 25 to 30 per cent of the ovules in each cross were not entered by the pollen tubes, and these ovules became a dry powder. Second, 10 to 13 per cent of the embryo sacs entered by the tubes failed to develop endosperm, in which cases both egg and polar nuclei degenerated probably because they were not fertilized; these ovules also became dry powder. Third, 8 to 9 per cent of the sacs showed disintegrating zygotes with the presence of developing endosperm, and later about 79 per cent showed arrested embryos in various stages of abortion.

With respect to the first result, that 25 to 30 per cent of the ovules were not entered by pollen tubes, this must be due to the slower growth of the tubes of franciscana in the style of the tetraploid and vice versa. Thus the tubes of franciscana practically all entered the sacs of franciscana within 48 hours but did not enter the sacs of the tetraploid until 96 hours. The tubes of the tetraploid entered the sacs of the tetraploid within 72 hours but did not enter the sacs of franciscana until 96 hours. It is clear that conditions within the styles of franciscana

and the tetraploid delayed by at least 24 to 48 hours the growth of pollen tubes of the opposite type. During this period of delay there was an aging of tubes and a withering of the styles and it is suggested that this may end the growth of many tubes before they have reached the ovules.

Bearing in mind the delayed growth of pollen tubes in these two crosses, it is possible that the responsibility for the second result, the failure of endosperm to develop in 10 to 13 per cent of the embryo sacs entered by the tubes, may lie in the over maturity of the sex nuclei. It was clearly shown in franciscana selfed that both egg and sperm nuclei were ready to function at from 48 to 72 hours after pollination. Since in the two reciprocal crosses a large percentage of the pollen tubes are delayed by 24 to 48 hours in reaching the sacs, both sperm, egg, and polar nuclei may become too old to function. If this is true, double fertilization can not result even though pollen tubes enter the sacs.

With respect to the third result, that 8 to 9 per cent of the sacs showed degenerating zygotes, even though they lay in developing endosperm, and

that later about 79 per cent of the ovules showed abortive embryos, the results may again be due to a reduced physiological vigor on the part of the sex nuclei held 24 to 48 hours over the usual time for their functioning. It seems probable that these gametes, even when they do fuse, either cannot develop viable zygotes or later fail to develop normal embryos. Hence embryos become arrested at various stages, resulting in such high proportions of sterile seeds as about 87 per cent for franciscana x tetraploid and about 88 per cent for tetraploid x franciscana.

SUMMARY

1. Oenothera franciscana and a tetraploid derivative breed very true and have a seed fertility of about 90 per cent and 60 per cent respectively. Their reciprocal crosses, however, produce surprisingly small number of fertile seeds, with an average germination of 13.5 for franciscana x tetraploid, and 11.9 per cent for the tetraploid x franciscana. The triploids (21 chromosomes), from these crosses show from 12 to 17 per cent of fertile seeds and segregate a wide range of types including frequent franciscana plants (14 chromosomes), and franciscana dwarfs (14 chromosomes), rarely triploids (21 chromosomes), occasionally tetraploids (28 chromosomes), and various hyperploids (15, 16, 22 chromosomes).

2. The chromosome configurations at diakinesis in the megasporocytes show for franciscana its 14 chromosomes in 7 pairs, and for the tetraploid its 28 chromosomes distributed as mixtures of quadrivalents in chains or in circles, bivalents in rings or end to end, and frequently univalents. The triploid presents its 21 chromosomes in univalents,

bivalents and trivalents in chains.

3. The chromosome segregation in the megasporocyte of franciscana is very regular, giving to each of the resulting nuclei 7 chromosomes. The tetraploid presents frequent irregularities due mainly to the lagging of the chromosomes often in the first division and occasionally in the second division. The highest degree of irregularities is found in the triploid due to two chief factors: firts, the varied arrangements of the trivalents at metaphase of the first division together with the chance distribution of the univalents; second, the lagging of chromosomes and non-disjunction especially in the first division. Segregation in the triploid gives nuclei with a range of from 7 to 14 chromosomes.

4. The lengthwise splitting of the chromosomes previous to the first meiotic division appears earlier in the megasporocytes of these plants than has been reported for the microsporocytes. In franciscana it becomes clear approaching late anaphase, and in the tetraploid it is evident as early as late prophase and conspicuous at diakinesis when distinct double threads connect the ends of chromosomes. In

the triploid the splitting appears during diakinesis, much earlier than in franciscana but later than in the tetraploid.

5. The female gametophytes usually develop from the megaspore nearest to the micropyle. They are tetranucleate. Occasionally twin gametophytes are present only one of which survives.

6. The pollen tube growth of franciscana is rapid reaching the embryo sacs about 48 hours after pollination. Some tubes of the tetraploid may take 72 hours to reach the sacs. Tubes of franciscana grow very slowly in the style of the tetraploid requiring in some cases 96 hours to penetrate the sacs and the tubes of the tetraploid grow even more slowly in the styles of franciscana.

7. The factors responsible for the tardiness of the pollen tube growth in the two crosses seem to be associated with abnormal quantitative chromosome relations between the tissue of the style and pollen tube.

8. When the two sperm nuclei are discharged one of them fuses with the egg nucleus and the other with the polar nucleus. The primary endo-

sperm nucleus may have produced 16 endosperm nuclei before the zygote prepares for the first division. Fertilization in franciscana takes place in all the sacs that have been entered by the pollen tubes and in the tetraploid fertilization is usually successful. In the two crosses 25 to 30 per cent of the sacs are never entered by the tubes, and in many cases fertilization does not follow after their entrance.

9. The endosperm growth and the first division of the zygotes are regular and normal in franciscana, but in the tetraploid certain amounts of abnormalities are found in these two developments. The two reciprocal crosses present very large numbers of zygotes and much endosperm in stages of disintegration.

10. The embryo development in franciscana is very uniform and irregularities were not noted until late in the development of the seeds about 5 per cent of which were found to contain abnormal embryos. In the tetraploid embryo development was found to be arrested at various stages from the beginning to the mature seeds with the result that

33.2 per cent of the seed-like structures had aborted embryos or were empty. In the two crosses many more embryos were found abortive at various stages of development; early breaking down resulting in empty seeds. In the cross franciscana x tetraploid about 87 per cent and in the reciprocal cross about 88 per cent of the seeds were found to contain abortive embryos or to be empty.

11. The high seed sterility presented by these two crosses is believed to be due (1) partly to the irregularities of chromosome segregation in the tetraploid, but (2) chiefly to the aging of the gametes because of the slow growth of the pollen tubes.

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EXPLANATION OF FIGURES

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 ocular K20. The magnification for all the figures was 2400 diameters. In reproduction they are reduced about one third.

Plate I.

Oenothera franciscana

Figure 1. Open spireme following synizesis. Loops expanding from a central region.
Figure 2. Emerging from second contraction. Constrictions beginning to appear.
Figure 3. Early diakinesis. 7 pairs of chromosomes.
Figure 4. Diakinesis. Chromosomes greatly condensed in 7 rings.
Figure 5. Metaphase of first meiotic division.
Figure 6. Late anaphase of first meiotic division. Seven split chromosomes at each pole.
Figure 7. Interkinesis. Seven split chromosomes in each nucleus.
Figure 8. Metaphase of second meiotic division. Seven split chromosomes on the equatorial plate.
Figure 9. Anaphase of second meiotic division. Seven chromosomes at each pole.
Figure 10. Telophase of second meiotic division. Seven chromosomes in each nucleus beginning to lengthen.

PLATE I

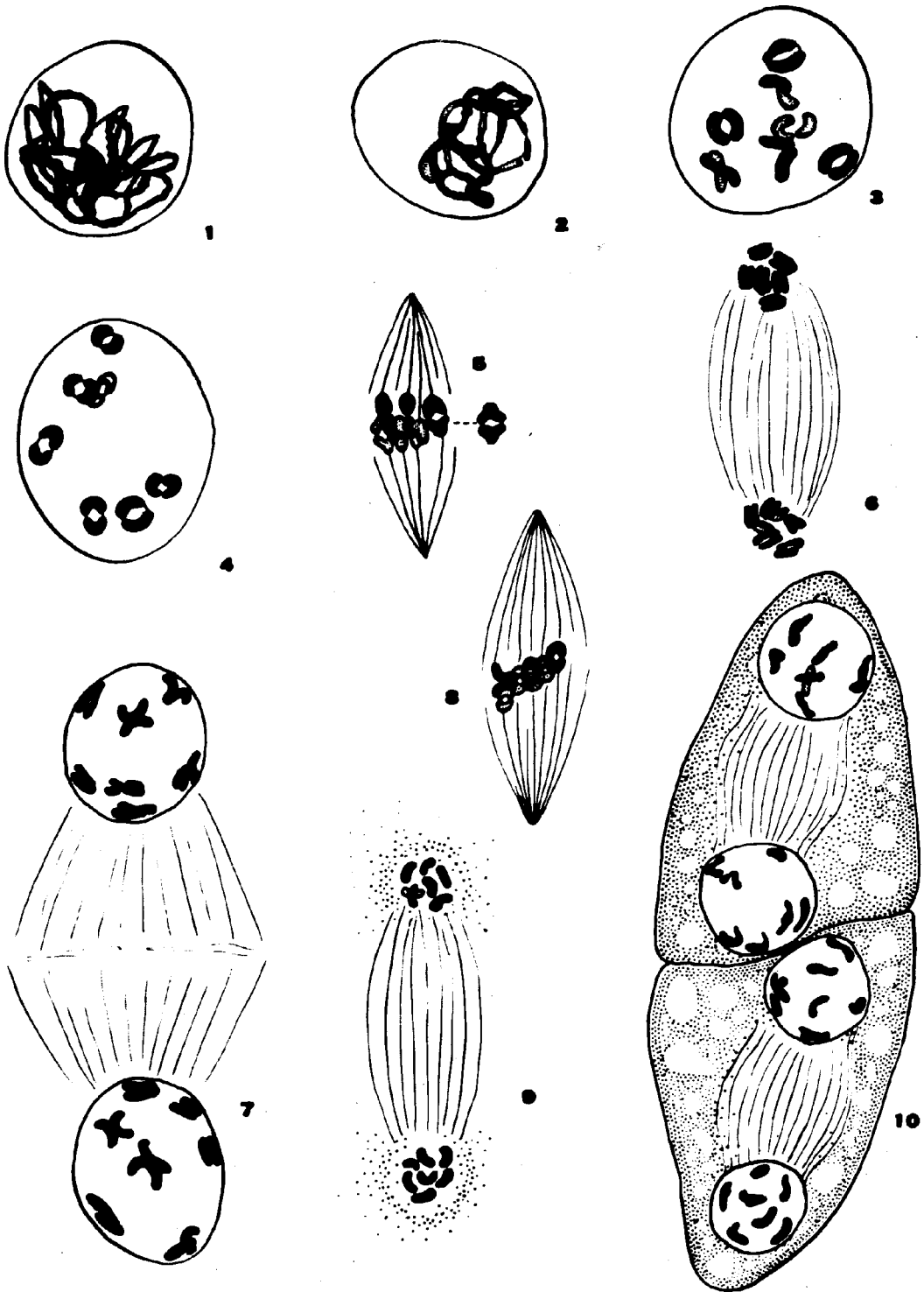


Plate II.

Tetraploid

Figure 11. Open spireme following synizesis. A much longer thread system than in *franciscana*.

Figure 12. Emerging from second contraction. Evidence of the lengthwise division in the chromosome segments.

Figure 13. Early diakinesis. Lengthwise division of the chromosomes is clear together with double connecting threads.

Figure 14. Diakinesis. Whole set of 28 chromosomes, in quadrivalents, bivalents and univalents.

Figure 15. Metaphase of the first meiotic division.

Figure 16. Interkinesis. 14 split chromosomes in each nucleus.

Figure 17. Metaphase of the second meiotic division.

Figure 18. Telophase of second meiotic division. 14 chromosomes in each nucleus in one diad. 13 chromosomes in each nucleus in the other diad with one split chromosome left between.

PLATE II

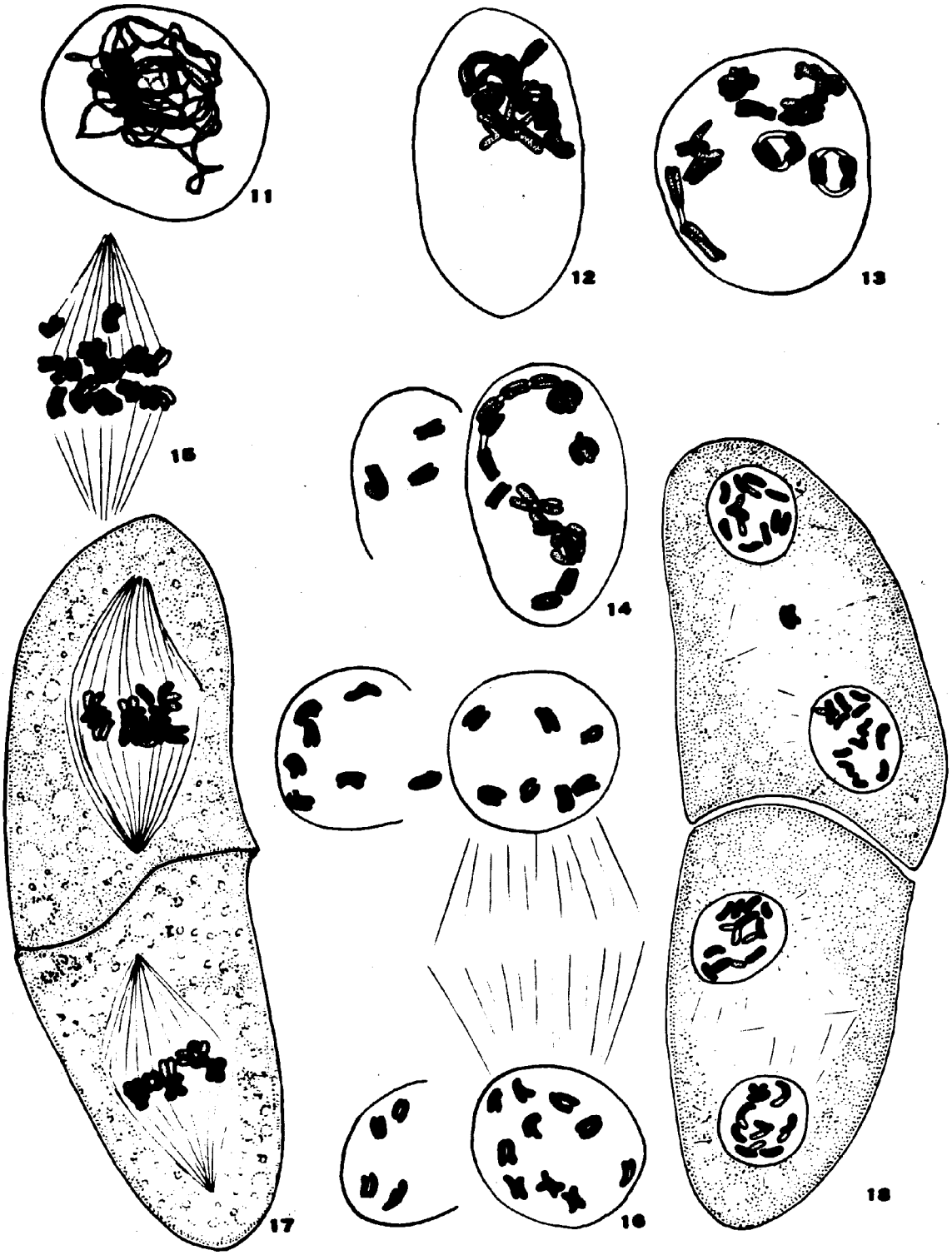


Plate III.

Triploid

Figure 19. Spireme entering second contraction.
Figure 20. Second contraction.
Figure 21. Late prophase. A lengthwise division of the chromosomes is indicated by the double connecting threads.
Figure 22. Diakinesis. 21 chromosomes in trivalents, bivalents and univalents.
Figure 23. Metaphase of the first meiotic division. 4 trivalents, 3 bivalents and 3 univalents are shown.
Figure 24. Anaphase of the first meiotic division. Chromosomes clearly split. Characteristic lagging chromosomes are shown.
Figure 25. Interkinesis. 9 split chromosomes in one nucleus and 7 in the other with 5 left between.
Figure 26. Metaphase of the second meiotic division with a small extra spindle concerned with two split chromosomes that lagged in the first division.
Figure 27. Late anaphase of the second meiotic division. One chromosome lagging.
Figure 28. Tetrad with a small extra cell.

PLATE III

