THE GENETICS AND CYTOLOGY OF TWO FIFTEEN-CHROMOSOME MUTANTS FROM THE HAPLOID OF *OENOTHERA FRANCISCANA* [1]

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INTRODUCTION

This report presents the results of cytological and genetical studies on two trisomic mutations occurring in *Oenothera.* It is hoped that the study will throw some light on the problem of the synaptic behavior in *Oenothera* and on the cause or causes of sterility of the particular form so commonly exhibited by chromosomal variants of the $2n + 1$ type. Inasmuch as a detailed statement of the pedigree and breeding data for each plant appears in tables 1 and 2, it will suffice here to make only a brief statement of the origin of the two variants.

Oenothera franciscana Bartlett, a wild species carried through fifteen generations by Davis, gave rise in 1923 to its first variant, a haploid called Pointed Tips (Davis and Kulkarni, 1930). Selfed seed of this haploid produced in 1924 (culture 24.25, table 1) a progeny of 329 *franciscana,* and in addition two more plants of the haploid. Seed from the 1924 haploids selfed, when planted in 1926 (culture 26.26, table 1), gave 55 *franciscana* and one plant dwarf like the haploid, but spreading and symmetrical in habit, quite rigid, and with broader leaves. This mutant was named Bushy Dwarf (fig. 1). In 1927, from seed of selfed haploids of 1926 (culture 27.41, table 2), there appeared, in addition to 107 of *franciscana* and two haploids (Pointed Tips), 13 dwarfs having long narrow leaves, a dense, low habit, and buds which were red and very long. Because of the character of the buds, this mutant was called Red Elongate (fig. 2). As shown in tables 1 and 2, the sports have arisen frequently in later cultures.

Since the two 15-chromosome mutants arose from seed of the 7-chromosome haploid selfed, the origin of the 8-chromosome gamete which must unite with the normal 7-chromosome gamete becomes a matter of interest. Davis and Kulkarni (1930) have shown that the small amount of fertile pollen developed by the haploid of *franciscana* results from the suppression of the heterotypic division to give a restitution nucleus which then goes through the homeotypic division to produce two pollen grains. Occasional

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non-disjunction in the homeotypic division (probably in the embryo sac) would give the 8-chromosome gamete necessary for the production of a 15-chromosome zygote. There may be seven possible types of 15-chromosome zygotes, since any of the 7 chromosomes of the *franciscana* set might be involved in non-disjunction. Such a group of seven trisomic forms would correspond to the twelve primaries established for *Datura* by Blakeslee and his co-workers.

Red Elongate and Bushy Dwarf differ from the parent, and in addition are distinctly unlike one another. Mutations of this type are known from

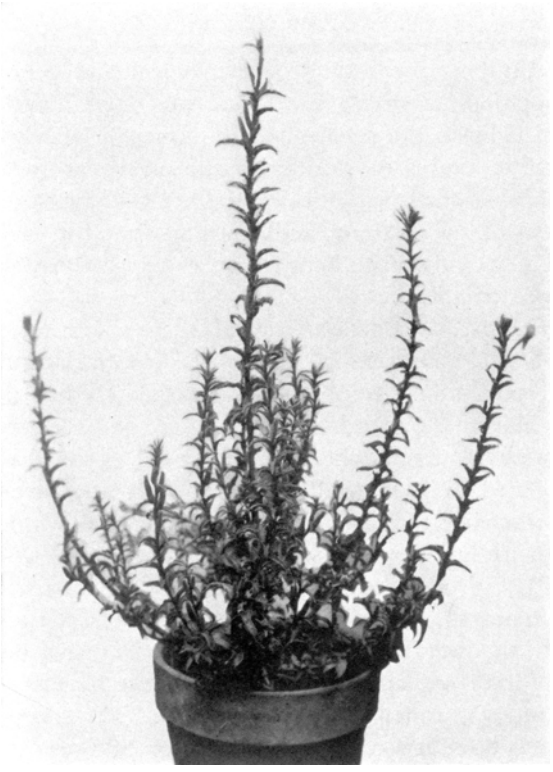

Fig. 1. Bushy Dwarf, 15-chromosome primary of *Oenothera franciscana.*

Datura Stramonium, where they have been studied in detail in the forms Globe and Poinsettia (Blakeslee, 1921; Blakeslee and Farnham, 1923). Clausen and Goodspeed (1924) in *Nicotiana* have found the sport Enlarged Flower to be of this character. Other forms are the Matthiola mutations of Frost and Mann (1924) and several sports in *Lycopersicum* (Lesley, 1928). In *Oenothera* trisomic sports are well known. Gates (1923), reviewing the literature of such trisomic forms, lists thirty-seven as having been reported from the time when the first chromosome counts of this kind were made in

1908 and 1909 for the *lata* and *albida* of de Vries. Håkansson (1930) has added to the list.

Red Elongate and Bushy Dwarf are non-chain-forming, since all of the chromosomes pair except the three that constitute the trisome and which for this reason are of particular interest. Because of the work of Weier (1930) on *Oenothera Hookeri,* in which parasynapsis was reported, it is to be expected that the same mode of synaptic behavior will be present in the progeny of such a closely related species as *Oenothera franciscana.*

That *Oenothera* exhibits telosynaptic behavior has been the contention of the majority of investigators. Gates (1907, 1909), Davis (1910, 1911), Geerts (1909, 1911), Cleland (1922–1929), Håkansson (1926, 1928), Sheffield (1927, 1929), Sinoto (1927), Illick (1929), and Kulkarni (1929) have

Fig. 2. Red Elongate, 15-chromosome primary of *Oenothera franciscana.*

been the foremost supporters of the telosynaptic interpretation. The list of the workers who hold to the parasynaptic interpretation includes Stomps (1910), Boedyn (1924, 1925, 1926), Schwemmle (1926), Lewitzky (1927), Kihara (1927), Darlington (1929, 1931), Weier (1930), Leliveld (1931), and Catcheside (1931a). Due to the work of Weier (1931), Darlington (1931), and Emerson (1931b), the opinions of *Oenothera* cytologists have probably in large measure shifted to the interpretation of parasynapsis. Gates and Goodwin (1931) have reported parasynapsis in *Oenothera purpurata* and *O. blandina* with seven pairs of chromosomes, and Catcheside (1931b) for a derivative from *Oenothera Lamarckiana* with a ring of 6 chromosomes and 4 pairs. Wisniewska (1932) gives evidence for parasynapsis in *Oenothera biennis* and in a form from *O. Hookeri* with a ring of 4 chromosomes and 5 pairs.

MATERIAL AND METHODS

The origin of Bushy Dwarf and Red Elongate is given in the Introduction, and tables 1 and 2 present in detail the breeding data for these mutations through later generations, while tables 3 and 4 give the results from back crosses of Bushy Dwarf and Red Elongate to the parent type *franciscana.*

TABLE 1. *Pedigree of Bushy Dwarf*

Culture	Parents	% germination	*O. franciscana*	Bushy Dwarf	Red Elongate	Pointed Tips	Misc. Dwarf	New types	Died	Total
24.25 (Davis)	23.21–165 (Pointed Tips) (Haploid)	94	329	0	0	2	1	0	29	361
26.26 (Davis)	24.25–332 (Pointed Tips) (Haploid)	68.2	55	1	0	0	2	0	2	60
27.43 (Davis)	26.26–40 (Bushy Dwarf) ($2n+1$)	53.4	135	21	1	0	1	0	6	164
28.36 (Davis)	27.43–57 (Bushy Dwarf) ($2n+1$)	26.2	63	8	0	0	0	0	8	79
29.44 (Davis)	27.43–57 (Bushy Dwarf) ($2n+1$)	53.6	186	32	0	0	0	0	12	230
30.35 (Davis)	29.44–215 (Bushy Dwarf) ($2n+1$)	81.1	232	26	0	0	0	0	12	270
31.41 (Anderson)	30.35–110 (Bushy Dwarf) ($2n+1$)	78.8	177	21	0	2	0	1	42	243
31.42 (Anderson)	30.35–112 (Bushy Dwarf) ($2n+1$)	76.2	187	22	0	0	0	2	20	231
32.17 (Anderson)	31.42–33 (Bushy Dwarf) ($2n+1$)	70.9	51	10	0	0	0	0	1	62
32.18 (Anderson)	31.42–199 (Bushy Dwarf) ($2n+1$)	62.07	18	4	0	0	0	0	0	22
32.19 (Anderson)	31.42–209 (Bushy Dwarf) ($2n+1$)	86.2	89	25	0	0	0	2	1	117
32.20 (Anderson)	31.41–61 (Bushy Dwarf) ($2n+1$)	78.4	106	40	1	0	0	0	3	150
32.21 (Anderson)	31.46–305 (Bushy Dwarf) ($2n+1$)	64.1	65	1	0	0	0	0	0	66
Total			1693	211	2	4	4	5	135	2055

TABLE 2. *Pedigree of Red Elongate*

Culture	Parents	% germination	*O. franciscana*	Red Elongate	Bushy Dwarf	Pointed Tips	Misc. Dwarf	New types	Died	Total
24.25 (Davis)	23.21–165 (Pointed Tips) (Haploid)	94	329	0	0	2	1	0	29	361
26.25 (Davis)	24.25–248 (Pointed Tips) (Haploid)	74	29	0	0	3	0	0	5	37
27.41 (Davis)	26.25–13 (Pointed Tips) (Haploid)	75	107	13	0	2	9	0	0	131
28.35 (Davis)	27.41–127 (Red Elongate) ($2n + 1$)	86.1	185	20	1	1	0	0	17	224
29.42 (Davis)	27.41–127 (Red Elongate) ($2n + 1$)	80.5	72	12	0	1	0	0	0	85
29.43 (Davis)	27.41–88 (Red Elongate) ($2n + 1$)	64.9	81	11	1	0	1	0	0	94
30.31 (Davis)	29.42–75 (Red Elongate) ($2n + 1$)	77	92	20	0	0	0	0	0	112
30.32 (Davis)	29.43–61 (Red Elongate) ($2n + 1$)	57.2	98	10	0	0	1	0	0	109
31.31 (Anderson)	30.31–88 (Red Elongate) ($2n + 1$)	45.8	98	4	0	0	0	1	29	132
31.32 (Anderson)	30.31–90 (Red Elongate) ($2n + 1$)	27.3	41	0	0	0	0	0	40	81
31.33 (Anderson)	30.31–107 (Red Elongate) ($2n + 1$)	74.7	114	2	0	0	0	1	20	137
31.34 (Anderson)	30.31–108 (Red Elongate) ($2n + 1$)	84.5	121	8	0	1	0	1	16	147
31.35 (Anderson)	30.32–45 (Red Elongate) ($2n + 1$)	86.3	252	15	1	3	0	0	42	313
32.01 (Anderson)	31.35–285 (Red Elongate) ($2n + 1$)	60.8	23	3	0	0	0	0	0	26
32.02 (Anderson)	31.35–103 (Red Elongate) ($2n + 1$)	87.8	178	1	0	0	0	0	1	180
32.03 (Anderson)	31.35–171 (Red Elongate) ($2n + 1$)	68.7	46	1	0	0	0	0	0	47
32.05 (Anderson)	31.33–108 (Red Elongate) ($2n + 1$)	81.2	227	3	0	0	0	0	3	233
32.06 (Anderson)	31.35–302 (Red Elongate) ($2n + 1$)	89.03	181	18	1	0	0	2	3	205
32.07 (Anderson)	31.34–142 (Red Elongate) ($2n + 1$)	73.26	45	3	0	0	0	0	0	48
Total			2319	144	4	13	12	5	205	2702

Cytological material was collected by Professor Davis in 1927, 1929, and 1930, and given to the writer for study. In addition to this material, the writer made large collections in 1931.

TABLE 3. *Reciprocal crosses between O. franciscana and Red Elongate*

Culture	Parents	% germination	*O. franciscana*	Red Elongate	Bushy Dwarf	Pointed Tips	Misc. Dwarf	Runts	New types	Died	Total
31.36 (Anderson)	(30.22–78 × 30.31–90) (*O. franciscana* × Red Elongate)	95.4	860	0	0	0	0	0	4	13	877
31.37 (Anderson)	(30.31–90 × 30.22–788) (Red Elongate × *O. franciscana*)	61.3	127	6	0	1	1	0	0	12	147
31.38 (Anderson)	(30.22–788 × 30.31–108) (*O. franciscana* × Red Elongate)	96.9	524	1	0	0	1	0	0	4	530
31.39 (Anderson)	(30.31–108 × 30.22–788) (Red Elongate × *O. franciscana*)	77.0	116	4	0	0	0	0	0	13	133
32.08 (Anderson)	(31.35–163 × 31.35–162) (Red Elongate × *O. franciscana*)	74.16	75	3	0	0	2	0	0	0	80
32.09 (Anderson)	(31.34–138 × 31.34–137) (Red Elongate × *O. franciscana*)	84.6	54	2	0	1	0	0	0	1	59
32.10 (Anderson)	(31.34–143 × 31.34–144) (Red Elongate × *O. franciscana*)	74.5	63	5	0	1	0	0	0	0	69
32.11 (Anderson)	(31.34–134 × 31.34–137) (Red Elongate × *O. franciscana*)	74.8	78	2	0	0	0	0	1	1	82
32.13 (Anderson)	(31.34–142 × 31.34–144) (Red Elongate × *O. franciscana*)	80.5	63	1	0	0	0	0	1	1	66
32.14 (Anderson)	(31.34–145 × 31.34–144) (Red Elongate × *O. franciscana*)	79.2	108	6	0	1	0	0	0	1	116
32.15 (Anderson)	(31.33–132 × 31.33–131) (*O. franciscana* × Red Elongate)	69.77	218	0	1	0	0	0	0	1	220
32.16 (Anderson)	(31.35–162 × 31.35–163) (*O. franciscana* × Red Elongate)	72.7	229	0	0	0	0	1	0	5	235

Bushy Dwarf appeared as 9.7 per cent of the total in the series of 13 cultures given in table 1, and Red Elongate as 5.3 per cent of the total of 19 cultures given in table 2. The death rate of seedlings in the cultures of Red Elongate (cultures 28.35–32.07, table 2) was 7.6 per cent; of Bushy Dwarf, 6.3 per cent (cultures 27.43–32.21, table 1). This seems to indicate greater viability of Bushy Dwarf seedlings, as germination rates were ap-

TABLE 4. *Reciprocal crosses between O. franciscana and Bushy Dwarf*

Culture	Parents	% germination	*O. franciscana*	Bushy Dwarf	Red Elongate	Pointed Tips	Misc. Dwarf	New types	Died	Total
30.36 (Davis)	(29.61–5 × 29.44–215) (*O. franciscana* × Bushy Dwarf)	96.9	286	0	0	0	0	0	0	286
30.37 (Davis)	(29.44–215 × 29.61–5) (Bushy Dwarf × *O. franciscana*)	59.2	119	15	0	1	0	0	0	135
31.43 (Anderson)	(30.22–788 × 30.35–110) (*O. franciscana* × Bushy Dwarf)	93.0	533	1	0	0	0	0	29	563
31.44 (Anderson)	(30.35–110 × 30.22–788) (Bushy Dwarf × *O. franciscana*)	80.8	200	4	0	0	0	1	22	227
31.45 (Anderson)	(30.22–788 × 30.35–112) (*O. franciscana* × Bushy Dwarf)	91.0	406	0	0	2	1	2	44	455
31.46 (Anderson)	(30.35–112 × 30.22–788) (Bushy Dwarf × *O. franciscana*)	90.5	248	36	0	2	1	1	34	322
32.22 (Anderson)	(31.42–145 × 31.42–144) (Bushy Dwarf × *O. franciscana*)	72.89	64	0	0	1	0	0	0	66
32.23 (Anderson)	(31.42–3 × 31.42–4) (Bushy Dwarf × *O. franciscana*)	56.9	19	3	0	0	0	0	1	23
32.24 (Anderson)	(31.46–315 × 31.46–228) (Bushy Dwarf × *O. franciscana*)	77.5	176	19	0	0	1	1	1	198
32.25 (Anderson)	(31.42–144 × 31.42–145) (*O. franciscana* × Bushy Dwarf)	77.7	245	0	0	0	0	0	0	245
32.26 (Anderson)	(31.41–60 × 31.41–61) (*O. franciscana* × Bushy Dwarf)	85.5	343	0	0	0	1	1	1	346
32.27 (Anderson)	(31.41–228 × 31.41–226) (*O. franciscana* × Bushy Dwarf)	84.9	197	0	0	0	0	0	0	197
32.28 (Anderson)	(31.41–238 × 31.41–237) (*O. franciscana* × Bushy Dwarf)	84.6	162	0	0	0	0	0	0	162
32.29 (Anderson)	(31.31–104 × 31.31–103) (*O. franciscana* × Bushy Dwarf)	94.6	321	0	0	1	0	0	0	322
32.30 (Anderson)	(31.35–294 × 31.35–297) (*O. franciscana* × Bushy Dwarf)	83.78	400	0	0	1	2	0	2	405
32.31 (Anderson)	(31.41–120 × 31.41–121) (*O. franciscana* × Bushy Dwarf)	95.6	212	0	0	0	0	0	1	213
32.32 (Anderson)	(31.44–204 × 31.44–226) (*O. franciscana* × Bushy Dwarf)	82.9	301	0	1	0	0	0	1	303
32.33 (Anderson)	(31.46–162 × 31.46–161) (*O. franciscana* × Bushy Dwarf)	87.08	762	4	0	0	2	0	3	771

proximately the same—68.7 per cent for Red Elongate and 71.5 per cent for Bushy Dwarf. This difference in the relative percentages of the appearance of the two sports is not entirely accounted for by differential seedling vigor. The number of dwarf rosettes and runts is twice as great in cultures of Red Elongate selfed as in cultures of Bushy Dwarf selfed, indicating an initial loss of Red Elongate forms through possible chromosome disarrangement.

In January, 1931, seeds subjected to freezing weather were germinated in Petri dishes following treatment of pressure and exhaust employed by Davis (1915). The cultures were from Red Elongate and Bushy Dwarf selfed and from both variants backcrossed to *franciscana*. Particularly large crosses were grown of the back crosses where Red Elongate and Bushy Dwarf had been used as the male parent (cultures 31.36, 31.38, 32.15, and 32.16, table 3; 31.43, 31.45, 32.27, and 32.33, table 4) to determine whether or not the high degree of male sterility was total. Germination following the treatment described above took place immediately, and the method has the advantage over earth-sown cultures in that it gives an accurate check on germination through the residue of ungerminated seeds that may be examined.

Cytological collections were made at all stages of development of anthers and ovaries. Anthers were fixed according to the following treatments, the dehydration being always through a long series of alcohols: (1) acetic acid-absolute alcohol (1:3), fixed not over 15 minutes; (2) Allen's modification of Bouin,[2] 6 hours, washed 24 hours; (3) strong Flemming, 24 hours, washed 24 hours; (4) Gilson's with 1 per cent acetic acid,[3] fixed 6 hours followed by 50 per cent, 60 per cent, 70 per cent, 85 per cent alcohols; (5) La Cour's (1929), 12 to 24 hours, washed 24 hours; (6) Nawaschin (Karpechenko),[4] 6 to 12 hours; (7) Telyesniczky, aqueous (Lee, 1928), 24 to 48 hours, washed 12 hours; (8) Zenker's, 6 hours, washed 12 hours; (9) Zirkle (1928), sulphuric acid-formaldehyde, various lengths of time up to 12 hours, thorough washing; (10) Zirkle,[5] formic acid-acetaldehyde-picric acid, fixation 48 hours, long washing. Material of ovaries was fixed in acetic-acid-absolute alcohol (1:3), Allen's modification of Bouin, strong Flemming, strong Flemming diluted one-half, Gilson's with 5 per cent acetic acid,[3] Nawaschin (Karpechenko),[4] Telyesniczky (aqueous), and La Cour's. Gilson's with 5 per cent acetic acid [3] was used most extensively, the fixation being for 6 hours, followed by treatment with 50 per cent, 60 per cent, 70 per cent, and 85 per cent alcohols. Whenever aqueous fixing fluids were used in treatment of anthers, it was found expedient to brush the anthers with water to hasten penetration.

[2] Allen's Modification of Bouin: Bouin's fluid, 100 cc.; chromic acid, 0.5 g.; urea, 2.0 g.

[3] Modified Gilson's with 1–5 per cent acetic acid: Bichloride of mercury (sat. sol. in 40 per cent alcohol), 100 cc.; nitric acid (c.p.), 2 cc.; glacial acetic acid, 1–5 cc.

[4] Nawaschin (Karpechenko): (*A*) Chromic acid, 4 g.; glacial acetic acid, 40 cc.; water, 240 cc. (*B*) Formalin, 160 cc.; water, 140 cc.

[5] Zirkle's formula (suggested through correspondence): Formic acid, 2 cc.; acetaldehyde, 6 cc.; picric acid (sat. aqueous), 92 cc.

In the case of ovaries an attempt was made to cut the material in pieces so small that penetration would be rapid.

Experience with mixtures so varied in nature impresses one with the fact that there is no fixing fluid uniformly superior for all phases of *Oenothera* meiosis. It was in general true that Nawaschin's [4] fluid gave rather better results in the fixation of anthers providing it was changed after the first hour. Gilson's with 1 per cent acetic acid, both from the view of relatively little shrinkage and brilliancy of staining following its use, rated next to Nawaschin's. Strong Flemming and Allen's modification of Bouin [2] were both satisfactory, but better differentiation was obtained in the contraction stages following treatment with the first two named. The acetic acid-alcohol (1:3) fixation proved rather disappointing, as staining after its use was unsatisfactory even if the sections were mordanted in Telyesniczky's fluid over night, as Weier (1930) suggests. Zirkle's sulphuric acid-formaldehyde fluid did not work at all well (Zirkle, 1928). His formic acid-acetaldehyde-picric acid was satisfactory but not better than the others, with the added difficulty involved in using the rapidly volatilizing acetaldehyde. Gilson's fluid with 5 per cent acetic acid proved most satisfactory in the case of studies of the ovary, although strong Flemming and Allen's modification of Bouin gave good results; but Flemming, diluted one-half, was very unsatisfactory. La Cour's (1929) fluid gave indifferent or erratic results; shrinkage following its use differed widely, depending on the phases studied in the sections treated. Shrinkage was evident in all material of both anthers and ovaries, regardless of the reagent, but varied greatly in degree.

Anthers were cut from 3 to 13 microns thick, the majority at 10 microns. Ovaries as a rule were cut at 11 microns but up to 20 microns when necessary.

Iron-alum-haematoxylin as usual was very satisfactory. For sections of ovaries thicker than 14 microns safranin counter-stained with light green according to the method of Blackman and Welsford (1913) proved useful. Crystal violet in 0.5 per cent aqueous solution according to the following schedule, Newton's modification of the Gram method, gave excellent results. After five to fifteen minutes in crystal violet the slides were rinsed in water, allowed a minute in 1 g. potassium iodide + 1 g. iodine in 100 cc. 80 per cent alcohol, and then passed very rapidly through 95 per cent and absolute alcohols. The preparations were cleared in a mixture of clove oil and xylol (1:3), where final differentiation took place, and followed by xylol to remove all of the clove oil.

No success attended attempts to germinate pollen in various concentrations of sugar. The addition of saliva or diastase to the media, as suggested by Tischler (1927), did not help. Sugar media with crushed stigmas were also tried, but without success.

THE CYTOLOGY OF RED ELONGATE

Somatic mitosis

Material excellent for studies on somatic mitosis was furnished by the anther walls and the nucellus of the developing ovule. The resting nucleus (fig. 1, pl. 22) has several nucleoli, only one of which is large. Within the nucleus is a fine network in which are distributed chromatic bodies. These bodies frequently number fifteen, which is the number of somatic chromosomes present. However, counts ranging from eleven to seventeen have been made; so it is not true that the number of these bodies consistently correspond to the number of chromosomes.

During prophase there is a rapid thickening of the threads (fig. 2, 3, pl. 22) which, however, does not proceed uniformly until the formation of the spireme has taken place (fig. 4). The crosswise segmentation of the spireme, partially indicated in figure 4, results in the formation of fifteen chromosomes, as shown in figure 5. This same figure shows lengthwise splitting of the chromosomes. These chromosomes are rod-shaped, of varying lengths, and somewhat bent at this stage. The chromosomes then move into the metaphase position, a polar view of which is given in figure 6. Still retaining the form of bent rods, the daughter chromosomes move to their respective poles (fig. 7), and the daughter nuclei are organized, with the chromosomes at first clearly evident (fig. 8). An early coarse network is then developed and is later replaced by a finer one, the nucleoli reappear, and the resting stage is again present (fig. 9).

Microsporogenesis

The pollen mother cells usually occur in two rows, but occasionally as a single row. The nucleus is slightly oval in outline and measures on an average 10.9 microns through the longer axis. Together with the large nucleolus are found deeply staining bodies numbering fifteen as a rule, but smaller counts are frequently met, and occasionally counts of sixteen and seventeen have been determined (fig. 10, 11, 12). Endonucleoli may be observed as in figure 16. A very fine network seems to be present from the earliest stages. This network does not stain very readily, but may be distinguished (fig. 10–13).

As the nucleus approaches prophase of meiosis, a thickening of the strands of the network takes place together with an elongation of the small oval bodies found in the earlier stages (fig. 13, 14). Later, spherical granules appear more or less uniformly distributed along the threads (fig. 15, 16, 17). The figures from 16 to 24 indicate a system of threads rather than a reticulum. This would suggest that the reticulate appearance in the earliest stages is due to the adsorption of stain at points where crossed threads have become joined in the process of fixation.

The thread system is at first made up of strands of uniform diameter

(fig. 16, 17), but later some threads appear to thicken at the expense of others which become attenuate. This finally results after synizesis in a relatively simple and much shorter thread system, as illustrated in figures 20, 21, and 22. While the thread system is still delicate, the nucleolus passes to the periphery of the nucleus, and the chromatic thread system contracts into the synizetic knot (fig. 19). Usually this knot is formed at the periphery of the nucleus on the side nearest the anther wall, but in many cases it is not. This would seem to indicate that other factors aside from the movement of the fixing fluids must be concerned in its position. The knot varies in density with different fixing agents. For example, fixation with acetic acid-absolute alcohol or careful fixation with Nawaschin's fluid leaves the knot least dense, while strong Flemming and Allen's modification of Bouin draw the threads into a dense mass. Telyesniczky's (aqueous) fluid left the nuclear structure so contracted that it was practically impossible to study. The same fluids act in about the same way on second contraction figures. As stated, the knot varies with the use of different reagents; but the fact that it does occur with all of the fixing fluids suggests that it is a natural process and not wholly an artifact, although it is intensified by poor fixation.

It is evident that the apparently reticular chromatic structure of the early phases gives way to a thread system. Sections cut at 13 microns show this rather clearly (fig. 20, 22). The thread system on emergence from synizesis is much shorter and consists of thicker threads that have lost their spherical chromatic bodies. Finally the thread system expands uniformly throughout the nucleus to give the stage of the hollow spireme (fig. 21). The history of prophase shows the consistent development of a thread system, and the earlier appearance of a reticulum (fig. 15) probably results from imperfect fixation by which crossed threads become united at points.

Looping of the spireme (fig. 20, 21, 22) is evident after synizesis, and figures 23–27 show that this looping becomes very conspicuous as second contraction approaches. Preparations sometimes show an apparently homogeneous mass of chromatin, but such conditions are certainly the result of imperfect fixation. Undoubtedly such a mass is made up of a thread system, but the cohesion of the threads is such that the system is hidden. That each of six loops represents a pair of chromosomes, while the seventh loop is made up of three chromosomes, is a logical interpretation. Such loops, as Weier (1930) has shown in *Oenothera Hookeri,* result from contraction of laterally paired threads and not from spireme segmentation. Gates and Goodwin (1931) apparently support Weier's conclusions in observations on meiosis in *Oenothera blandina.* Interlinked simple rings do not appear in either Red Elongate or Bushy Dwarf as they do in both *blandina* and *purpurata.* The chromosome pairs are free from the earliest observations possible after emergence from the second contraction.

Evidence for parasynapsis from chiasma formation is clearly indicated in figures of Catcheside (1931b). Crossing-over has been established by

Shull (1930) between the form *old gold* and *bullata*. Emerson (1931b) found crossing-over between homologous parts of dissimilar chromosomes in a circle of ten in *franciscana-sulfurea*. If crossing-over is correlated with chiasma formation and chiasma formation with parasynaptic behavior, there is reason to expect that further direct evidence for parasynapsis will be found.

Stages of diakinesis were numerous and easily studied. Figures 30 and 31 illustrate configurations of seven pairs and a univalent and six pairs and a trivalent, respectively. The pairs occurring at diakinesis are of two types. In one there is only a connection at one end (fig. 30 *a*), while in the other (fig. 30 *b*) there is one at each end, making of the two chromosomes what Darlington terms a "simple ring." When these pairs become arranged on the spindle at metaphase, the first condition gives rise to the dumbbell form *a* and the second to the shield form *b*, as shown in figure 33. Counts of a large number of diakinesis figures established the fact that the usual arrangement consisted of 4 simple rings, 2 horseshoe-shaped figures, and a trivalent or 4 simple rings, 3 horseshoe-shaped figures, and a univalent. At metaphase this same formation was carried out with the appearance of 4 shield-shaped pairs, 2 rods, and a trivalent or 4 shield-shaped pairs, 3 rods, and a univalent. That is, the shield-shaped pairs result when the spindle fibers are attached to the mid-section of the chromosome, and the rod- or dumbbell-shaped pairs when the point of attachment of the spindle fiber is near terminal.

Multipolar spindles (fig. 32) precede the bipolar structure at metaphase. The chromosomes move from diakinesis to metaphase without changing form, so that rings, pairs, univalents, or trivalents may all be in evidence at metaphase. The more common configuration is that illustrated by figure 33.

During anaphase the odd chromosome of a trisome accompanies one of its homologues. If the odd chromosome is present as a univalent, it frequently lags and usually lies with its longer axis at right angles to the spindle. Ordinarily there is only one lagging chromosome (fig. 34), although occasionally two were observed. Such chromosomes apparently do not undergo division, or if they do, there is no separation of the halves. No disintegration was noticed, rather a loss in staining capacity. No evidence was found indicating that chromosomes were ejected into the cytoplasm outside of the spindle.

The first evidence of lengthwise splitting of the chromosomes appeared during telophase (fig. 36, 37), no split being observed during anaphase (fig. 35). The split becomes very pronounced during interkinesis (fig. 38, 39, 40).

As indicated in the foregoing account, the distribution of chromosomes is 7 and 8 where no lagging takes place. No 6 and 9 distributions were found, although a great many nuclei were studied. During interkinesis the halves of the split chromosomes draw away from each other at the ends, giving to each pair the appearance of a Maltese cross (fig. 39, 40). Later the chro-

mosome halves become more attenuate (fig. 41). Counts made at interkinesis bear out the statement regarding the frequency of lagging. Of 384 nuclei examined in the stage of interkinesis, 8 chromosomes appeared in only 135 nuclei instead of the expected 192. Care was taken to make this count in such pairs of nuclei as occurred in the same pollen mother cell.

The homeotypic division of meiosis takes place quickly. Figures 42 and 43 show two metaphases from the same sporocyte with 7 and 8 chromosomes, respectively. In figure 44 an early anaphase and a polar view of metaphase are shown. Lagging in the second division anaphase also occurs. Figure 45 presents a type that is common; the halves of a split chromosome fail to separate and degenerate in the central region of the spindle. This later lagging during the homeotypic division naturally results in further lowering the final number of 8-chromosome microspores formed. During telophase a chromatic network is developed through the elongation and anastomosing of the chromosomes (fig. 46). There was no indication of polyspory. Pollen grains were of two kinds, the good ones plump and well filled, the bad ones shrunken and finally without cell contents. Among the shriveled grains there were some not so much shrunken and possessing four instead of three lobes (fig. 88). These are believed to result from cells containing 8 chromosomes. The percentage of bad pollen varied slightly with different plants, but apparently not with the season, whether early or late. The largest proportion of bad pollen recorded was 58 per cent and the lowest 43 per cent.

Megasporogenesis

The megaspore mother cell lies deeply imbedded in the nucellus and becomes evident about the time when the integuments begin to form. The resting nucleus (fig. 47) exhibits one or more nucleoli and a variable number of chromatic bodies in a delicate reticulum. Some of these bodies are probably chromosome centers, but the number is not at all times in accord with the count of chromosomes in somatic tissues. A delicate thread system then develops (fig. 48), the material of the chromatic bodies apparently passing into this reticulum. This stage is soon followed by the stage of synizesis (fig. 49), which differs from the comparable figure in microsporogenesis in that the knot is less dense in structure.

Figures 50, 51, and 52 illustrate emergence from synizesis, with the formation of a thick spireme rather evenly distributed through the nucleus. A contraction of the spireme then takes place (fig. 53–56), but is not so marked in character as in the corresponding stage in the pollen mother cell. Darlington (1931a) suggests that the extreme contraction in *Oenothera* is due to the singleness of structure of the thread. However, the structure of the thread in nuclei of both micro- and megasporocytes is the same, and yet the degree of contraction is markedly different. The explanation of the contraction must then involve other factors. Following the apparent segmentation of the thread, the stages of diakinesis appear as illustrated in figures

57 and 58. It is interesting to note that the simple ring structures which appeared so often in microsporogenesis occur relatively infrequently. As a result the chromosomes at metaphase and anaphase (fig. 59–62) are found usually as rod-shaped pairs evidently developed from the horseshoe-shaped bivalents of diakinesis. A discussion of the matter will be presented later.

Counts of chromosomes made at diakinesis and at later stages established the number as fifteen. Figure 63 shows a 9–6 distribution of chromosomes which was not observed in the heterotypic division in microsporogenesis, but which is not infrequent here. Figures 64 and 65 illustrate a 7–7 and an 8–7 distribution, respectively. Lagging of chromosomes is usual, but only about half as frequent as in corresponding stages of microsporogenesis. The splitting of the chromosomes during interkinesis and the subsequent formation of X-shaped figures is less evident than in microsporogenesis. Interkinesis is of short duration and is often so rapidly followed by the homeotypic division that a wall between the dyads is not formed before the second division is well under way (fig. 66, 67, 68). Figure 68 shows a form of lagging frequently observed where a chromosome lies close to a daughter nucleus, having failed to become incorporated in it.

The final result of these two divisions is the formation of a tetrad of contiguous cells but of three distinct types. In some no walls appear between the megaspore nuclei (fig. 70); in others the walls are thin (fig. 71); and in a third type the walls are of considerable thickness (fig. 69, 73). The thick walls may possibly prevent fertilization of the embryo sac and subsequent seed development, resulting in some loss of fertility. These walls would not, however, act selectively against a particular type of megaspore, since all cells in the tetrad have the same heavy walls. Hence the wall formation has no significance in connection with the type of sterility found in these variants.

Only one megaspore nucleus survives, the other three breaking down. Ishikawa (1918) described the degeneration of the two center cells before their complete formation and the later degeneration of either the chalazal or micropylar cell. In this material no degeneration takes place until after all four cells are formed. In a count of 81 young embryo sacs 55 had developed from the cell at the chalazal end and 26 from that at the micropylar end. Occasionally the spores at both ends begin to form embryo sacs simultaneously, but only one continues development to maturity.

The surviving megaspore nucleus then moves to the micropylar end of the cell which is to become the embryo sac, and the lower portion of this cell becomes vacuolate (fig. 74). A group of 4 gametophyte nuclei is formed by two successive divisions giving rise to a pair of synergids (*s*), an egg nucleus (*e*), and a polar nucleus (*p*), as seen in figure 75. Usually the polar nucleus is the largest of the four. Large vacuoles appear between the synergids and the egg nucleus.

THE CYTOLOGY OF BUSHY DWARF COMPARED WITH RED ELONGATE

The studies made on Bushy Dwarf were as detailed as were those on Red Elongate. Morphologically the variants differed greatly, as shown in part by figures 1 and 2. Cytologically, however, meiosis in one runs parallel to that of the other, so much so that to present a complete series of figures for Bushy Dwarf would be a repetition of figures given for Red Elongate. Therefore only a few figures of stages representative of the similarity of the development will be presented.

The resting nucleus in the microsporocyte (fig. 76), like that in Red Elongate, contains several nucleoli and a delicate reticulum in which are imbedded chromatic bodies varying in number. Early prophase stages following the resting condition show the reticulum much more distinctly, one nucleolus and the gradual disappearance of the chromatic bodies. The disappearance is associated with the incorporation of the chromatin into the strands of the network (fig. 77). As synizesis approaches, the thread system becomes more marked, granular bodies appear on the threads, the nucleolus moves to the periphery, and a gradual contraction of the thread system takes place (fig. 78). On emergence from synizesis there is a thickening of the threads and a gradual filling of the nucleus with a system of threads now uniform in diameter, accompanied by a movement of the nucleolus toward the center of the nucleus (fig. 79). Later the threads thicken and become looped until at second contraction there is evident a configuration consisting of a relatively dense mass from which radiate loops, usually seven in number (fig. 80). Diakinesis figures which follow second contraction show as a rule a linear arrangement of the members of the trisome and six pairs of chromosomes. Occasionally the members of the trisome form a closed ring (fig. 81). In about half of the material seven pairs and a univalent were found. The multipolar spindle figure following diakinesis was again observed as in Red Elongate. Here again there is a preponderance of the shield-shaped chromosomes, indicating that at diakinesis the bivalent chromosomes occur more frequently as simple rings. Anaphase figures confirm the chromosome count of fifteen made in the earlier phases (fig. 83, 84). A lagging chromosome lying at right angles to the spindle fibers is illustrated in figure 84. This is the position which even at metaphase marks a chromosome as one which will lag. An attempt to relate the number of times that lagging took place to the occurrence of the odd chromosome as a univalent at diakinesis showed no correlation. The number of times the univalent lay at right angles to the spindle fibers at metaphase position was very closely correlated with the amount of lagging observed. Interkinesis figures in the two variants are similar (fig. 85), as are the homeotypic divisions which follow rapidly after interkinesis. This is illustrated by figure 86. The lagging of chromosomes during the homeotypic mitosis again reduces the numbers of 8-chromosome microspores. As in Red Elongate, polyspory was not observed.

Megasporogenesis in the two sports follows the same line of development

with two minor exceptions. Thick-walled tetrads were much less in evidence in Bushy Dwarf than in Red Elongate. This may account in part for the better seed production in Bushy Dwarf, since the presence of the thick walls may act as a deterrent to the fertilization of the embryo sac. The second peculiarity is the fact that in Bushy Dwarf the micropylar megaspore apparently functions with the same frequency as the chalazal megaspore in the development of the embryo sac, whereas in Red Elongate the latter seemed to be favored.

DISCUSSION

Several years ago Blakeslee (1921) outlined some results from work done on *Datura Stramonium,* and expressed the hope that certain of the findings might be helpful in the solution of the more difficult problems involved in the studies on *Oenothera.* The mutant called Globe appeared as the first of a series of forms differing markedly from one another and from the parent *Datura Stramonium.* After cytological study had established the fact that the peculiarities of the Globe variant were associated with the presence of an odd chromosome, the assumption was made that eleven more sports should make their appearance, due in each case to the duplication of a different chromosome of the 12 in the haploid set. This assumption was sustained by the later appearance of these eleven additional variants readily distinguished by morphological peculiarities. All of the twelve mutants were found to produce gametes with 12 and 13 chromosomes; bad pollen grains appeared in relatively large proportions; rarely was the new character transmitted through the pollen and only to about one-fourth of the offspring through the egg. The explanation of 12 distinct variants necessitated the further assumption that it was not only the presence of an odd chromosome which brought about the mutation, but that a duplication of a specific chromosome of a set was essential for the expression of a particular mutation complex. Fortunately, although the number of Mendelian characters with which *Datura* is supplied are few, sufficient of these were found to make possible a check on the mode of inheritance and on the chromosome carrier.

After the discovery of the twelve expected trisomic variations additional ones appeared, thus complicating what was apparently a simple situation. This resulted in the division of this type of variant into what Blakeslee terms primaries and secondaries. The primaries are distinguished from the secondaries, according to Blakeslee (1924), on the basis of both breeding behavior and the structure of the chromosomes. An example of breeding behavior is the fact that primaries occur spontaneously with much greater frequency than do secondaries. Furthermore, while secondaries occasionally come from their primaries, the latter arise regularly from their secondaries. The structure of the odd chromosome in the secondaries is believed to involve, as a rule, the duplication of a part. In one case (Blakeslee, 1924) the secondary resulted from the deficiency of a part. If the chromosomes of the trisome are *AB, AB, AB,* synapsis should show a pair and a univalent or a

chain of three. Should there be through segmental interchange a duplication and loss such that the chromosomes are *AAB*, *B*, *AB*, a ring might result as in secondaries. By a return of the segment *A* to the *B* chromosome the secondary would reproduce the primary *AB*, *AB*, *AB*. An instability of the *AAB*, *B*, *AB* condition may explain why secondaries throw back large proportions of primaries. Although this behavior would not involve the segmental interchange between non-homologous chromosomes (Belling and Blakeslee, 1924; Belling, 1925, 1927), the principle of the transfer of a portion of one chromosome to another is much the same. In *Datura* this duplication or deficiency of chromosome parts would account for the fact that secondaries appear to be modifications of their primaries. The peculiarities of the secondaries, according to Blakeslee, are not due to Mendelian factors.

In addition to secondaries, $2n + 1 + 1$ and $2n + 1 + 1 + 1$ forms have appeared in *Datura*—that is, two or more duplications of members of the haploid set have occurred. Theoretically this duplication might take place until all of the combinations between the diploid ($2n$) and triploid ($3n$) materialize. In *Datura* there have not been found forms with more than three additional chromosomes. McClintock (1929) in *Zea Mays* has reported duplication of as high as seven chromosomes of a haploid set of ten. These forms show an increase in the abnormal behavior with each additional odd chromosome, and this fact has an important bearing on the subject of meiotic irregularities met with in Red Elongate and Bushy Dwarf. The same increase in irregularity also has been observed in the odd chromosome variants of *Datura*.

As early as 1921 Blakeslee suggested that the variants of the Globe type had counterparts in the *lata* type of mutant found in *Oenothera*, with the exception that no chromosome characteristics of size or form have been found to show that the peculiarities of mutant *lata* are due to the presence of an odd chromosome of a particular set. Each of the two variants, Red Elongate and Bushy Dwarf, exhibits the odd chromosome, and the mutant character is transmitted as in *Datura* variants but in lesser degree. There is apparently complete male sterility in the *Oenothera* sports with respect to the $n + 1$ gamete, while transmission of the mutant character through the pollen of the Globe variant of *Datura* occurs in two per cent of the cases. Furthermore, there is a difference in the amount of bad pollen formed. While bad pollen in *Datura* variants is 7.9 per cent in the Globe and reaches only to 20.7 per cent in the variant Spinach, in Red Elongate and Bushy Dwarf the shriveled pollen ranges from 43 to 58 per cent.

The relation between nuclear disturbance and sterility has been established. A greater viability of n spores over $n + 1$ spores may be expected and less irregularity in the meiotic behavior of $2n + 1$ than in $2n + 1 + 1$ sports. Nuclear disturbance also may be comparatively greater in such variants as Red Elongate and Bushy Dwarf, in which one duplicate chromosome is added to the basic seven of the haploid set, than in *Datura* primaries, in which the addition of the odd chromosome is to a haploid set of twelve.

A corresponding difference exists between the number of $n + 1$ megaspores functioning in *Datura* sports and those in Red Elongate and Bushy Dwarf. In Globe the mutation complex in selfed forms is transmitted to about 25 per cent of the offspring and in Poinsettia to about 30 per cent. In the forms Red Elongate and Bushy Dwarf the transmission is to 15.1 and 19.4 per cent, respectively. Selective action against the $2n + 1$ zygote has been held responsible for some of the deficiency, but the difference in expected ratios may be associated with a possible greater disturbance brought about by the addition of the extra chromosome to the smaller seven-chromosome set of *Oenothera.* The difference in the greater viability of the $n + 1$ megaspore over the $n + 1$ microspore is perhaps attributable to the fact that its nutrition is better, and because it is better protected from the disturbance of environmental factors.

From the similarity of the behavior of the two variants to that exhibited by the *Datura* sports it seems quite possible that five more primaries will arise from *Oenothera franciscana* in addition to Red Elongate and Bushy Dwarf, since there are seven chromosomes in the haploid set. It may also be assumed that secondaries will occur such as have been found in *Datura.* The appearance of variants in which there is a duplication of more than one chromosome of the haploid set as in *Datura* is more problematical. The suppression of the $n + 1$ microspores seems to be complete; so any $2n + 1 + 1$ form would have to come from the fertilization of an $n + 1 + 1$ egg by a normal male gamete. Although a 9–6 chromosome distribution in megasporogenesis is not uncommon, no 16-chromosome variant has yet been isolated. The first appearance of Red Elongate and Bushy Dwarf was from the selfing of a haploid. Since $n + 1$ male gametes are apparently not formed, it may be assumed that the variant in each case resulted from the fertilization of an $n + 1$ female gamete by an n-chromosome male gamete. The $n + 1$ egg resulted from non-disjunction in the homeotypic division of the haploid following the suppression of the heterotypic mitosis. The incidence of mutation is about 1:1200 for Red Elongate and 1:2000 for Bushy Dwarf, as indicated by the occurrence of these sports when the cultures arise from *franciscana* pollinated by the variants. As far as the two variants are concerned, the writer is inclined to hold with the opinion expressed by Bartlett (1915) that in *Oenothera* no $n + 1$ male gamete functions. An apparent exception to this principle was encountered in culture 31.38, table 3, and culture 31.43, table 4, in each of which a single mutant appeared, the only cases out of some 3000 recorded for the back-cross, *franciscana* pollinated by the $2n + 1$ variants. It is possible that these two mutants were derived from $n + 1$ eggs of *franciscana,* the result of non-disjunction.

Buchholz and Blakeslee (1930) attribute the gametic sterility in primary mutations of *Datura* to selective action at germination of the pollen, selective action against the $n + 1$ gamete as exhibited by the differential rate of pollen tube growth, and bursting of pollen tubes, presumably those carrying the

$n + 1$ gametes. This would place the agencies responsible for sterility in the group of environmental factors. Goodspeed (1929) had already suggested that the production of fertile gametes in plants depends on nicely balanced physiological factors arranged in a particular manner in the living organism, and that numerous agents, either environmental or inherent, may upset this balance. Watkins (1932) has prepared a table of style and pollen tube chromosome relationships in various material which would indicate that the elimination of $n + 1$ gametes takes place due to faulty adjustments. That is, so long as the chromosomal relation between the stylar tissue and that of the pollen tube remains $2n$ and $1n$, there are no difficulties. Just as soon as the relation becomes as $2n$ and $1n + 1$, maladjustments result in selective action against the $n + 1$ pollen tube. It does not seem to the writer necessary to go beyond the actual formation of pollen grains in these two variants of *Oenothera* to account for the sterility, which involves, not the functioning of the $n + 1$ male gamete, but its non-formation.

The facts regarding extensive lagging of chromosomes have been given. Counts do not show the elimination of every 8-chromosome microspore prior to the tetrad stage, and the appearance of 4-lobed grains (fig. 88) somewhat larger than the normal grains seems to be significant. These pollen grains are found collapsed among the bad pollen grains when free pollen is examined (fig. 89). Harrison and Blackburn (1927) in their studies on pollen of roses found just such conditions obtaining in pollen with additional chromosomes. Michaelis (1926) in his work on *Epilobium angustifolium* found a relationship existing between chromosome number and morphological structure. The 4-lobed grains may then be those with the eight chromosomes.

Attempts to germinate *Oenothera* pollen by artificial means were not successful. Observations of stigmas which had been uniformly pollinated showed the germination of all good grains, indicating that if there were any 8-chromosome pollen grains among them they were not to be recognized through failure to germinate.

Referring back to the statement of Buchholz and Blakeslee (1930) that selective action at germination of pollen is one of the causes of sterility, it might be expected that delayed growth of pollen tubes of Red Elongate and Bushy Dwarf would be more pronounced than in *Datura* variants, since the odd chromosome is added to the smaller set of seven in the *Oenothera* sports instead of to the twelve in *Datura*. However, the apparent absence of the $n + 1$ male gamete in the two variants dealt with in these studies appears more likely to be explained by the non-formation of a large number of possible $n + 1$ microspores because of the lagging of the chromosomes and to the abortion of any 8-chromosome microspores, probably represented by the 4-lobed pollen grains.

The limited number of 8-chromosome megaspores in *Oenothera* is quite marked, the percentages for Red Elongate and Bushy Dwarf being 15.1 and 19.4, respectively. These figures were obtained by taking the ratio of the

total number of such variants appearing through selfed plants to the total number expected. In contrast with these percentages, Gates (1923) reported fertility as high as 50 per cent in his $2n + 1$ variants. Some of this low fertility may be accounted for by selective elimination of the $2n + 1$ zygote, and perhaps some is due to similar action against the female $n + 1$ gamete. Most of it is attributable to the loss of the odd chromosome in the meiotic processes. Lagging, although frequently met with in megasporogenesis, is not as extensive as it was in microsporogenesis.

The spindle fiber attachment to the chromosomes at metaphase of megasporogenesis was usually not at the mid-region, as had been the case in microsporogenesis. Odd chromosomes with the mid-region attachment were the ones which failed to be incorporated in the daughter nuclei at heterotypic division. Whether or not this may be taken as an indication of the loss of homology in the odd chromosome such that it fails to pair with its former homologues presents an interesting question. It is assumed in *Datura* that the odd chromosome may be modified either by duplication of a part or a deficiency of a part such that mutants known as secondaries result. A modification might be such as is represented by a change in the point of attachment of the spindle fibers. This theory of a change that will modify the degree of attraction between homologous chromosomes offers an hypothesis for the difference in the output of $n + 1$ microspores and $n + 1$ megaspores in *Oenothera*. The very dense second contraction figure of microsporogenesis offers a much greater opportunity for structural modification than does the looser figure found in the corresponding stage of megasporogenesis. On this basis, then, the odd chromosome in microsporogenesis which has its spindle fiber connection at the mid-region may be interpreted as one which has changed to such an extent that it fails to move to the pole, but remains at its metaphase position. The type of univalent having a terminal connection with its spindle fibers is apparently capable of becoming one of the members of the set of the daughter nuclei, at least during the dyad stage. It was not possible in the studies made to trace such a chromosome through the homeotypic division where lagging occurs, but it may be assumed that whatever lagging does take place involves one of the six halves of the members of the trisome which appeared at heterotypic metaphase.

Parasynapsis as the mode of meiosis has been assumed. Weier (1930), from studies of meiosis in *Oenothera Hookeri* with seven pairs of chromosomes, reports zygotene threads which, becoming shorter and thicker during pachynema, were counted as seven in number. During second contraction they appear as seven arms radiating from the central coagulum and are to be considered as bivalents resulting from parasynapsis. He concludes that *Oenothera Hookeri* presents meiotic behavior parasynaptic in character. In *Oenothera Lamarckiana* Weier finds pairing only between two threads representing, as he suggests, the single pair of chromosomes which appears at diakinesis in this species. The other twelve chromosomes are represented by

twelve free threads at this stage which become later a chain of twelve chromosomes. Gates and Goodwin (1931) present corroborative evidence of lateral pairing of threads from studies of meiosis in *Oenothera blandina* and *Oenothera purpurata,* two species exhibiting seven pairs of chromosomes, as in *Oenothera Hookeri.* These investigators conclude that the spireme, hitherto interpreted as being continuous, is not in that condition at any time in forms with paired chromosomes. The apparent continuity of the spireme is interpreted as due to the fact that interlinking of chromosome pairs takes place. The loops appearing at second contraction represent such interlocking, each loop consisting of a chromosome pair. If the figures descriptive of heterotypic mitosis in Red Elongate and Bushy Dwarf are compared with those presented by Weier (1930) and Gates and Goodwin (1931), the similarity is marked. Weier's " free arms " and the loops described by Gates and Goodwin are the same structurally. Evidence of conjugation is difficult to obtain, but in addition to that presented above, Emerson (1931b) shows presynaptic conjugation of pachynemal threads in an unknown species of *Oenothera,* a species having pairs instead of chains of chromosomes.

Darlington (1931), enlarging on his investigations of an earlier date (1929), presents another type of evidence for parasynaptic behavior in *Oenothera.* Assuming that studies on phases earlier than diakinesis meet with unusual technical difficulties, he reinvestigated the later stages. The appearance of double connections between the homologous ends of chromosomes reported by him indicates the terminalization of chiasmata between halves of the chromosomes. These chiasmata were formed in stages prior to diakinesis, indicating the presence of chromatids in those stages, a condition characteristic of parasynapsis. The two connections have not been frequently observed, possibly because they may fuse when imperfectly fixed and appear as one strand. A study of trivalents supports Darlington's conclusions, since, when trivalents occur as Y-shaped groups, the triangle formed by the connections does not collapse readily. Håkansson (1930) has found such conditions in the trisomic forms *curta* (a secondary from *cana*) and mutant *lata,* both *lata* and *cana* being mutants of *Oenothera Lamarckiana.* Emerson (1931a) presents, in his figures 287–6, 288–81, and 287–5 of an undetermined *Oenothera,* terminal connections which give evidence of earlier chiasma formation. In Red Elongate and Bushy Dwarf the presence of the trivalent offers no assistance, since it occurs in a chain form and not as a Y-shaped group, so that figures such as Håkansson (1930) presents in his studies are not possible.

Catcheside (1931b) gives the most convincing evidence of chiasma formation in studies on a form having a ring of 6 chromosomes and 4 pairs and believed to be a half-mutant from *Oenothera Lamarckiana.* The form shows lateral pairing, both in the chromosomes of the ring and in the free pairs. The difference in the pairing seems to be one of degree—that is, the chromosomes of the free pairs lie together along the entire length, while those

in the rings associate only at homologous segments. Demonstrations of chiasma formation were obtained beginning with late diplotene and in diakinesis and the heterotypic metaphase.

The above evidence for chiasma formation, together with the demonstration of crossing-over in *Oenothera* by Shull (1930) and Emerson (1931b), presents a strong argument for a parasynaptic interpretation of meiosis in *Oenothera*. If chiasmata are formed as the mechanism for crossing-over and are characteristic only of parasynaptic behavior, it is probable that the method of meiosis in all Oenotheras will be found to be parasynaptic.

SUMMARY

1. The appearance of two primary trisomic variants, Red Elongate and Bushy Dwarf, is reported from an *Oenothera* with pairing chromosomes. The sports originated from the selfing of a haploid (Pointed Tips), which in turn had its origin as a sport from *Oenothera franciscana.*

2. Bushy Dwarf appeared in the ratio of 1:422 from the haploid selfed (cultures 24.25 and 26.26, table 1), and Red Elongate appeared in the ratio of 1:41 from similar selfing (cultures 24.25, 26.25, and 27.41, table 2). In crosses in which the variants furnished the pollen with *franciscana* as the female parent (tables 3 and 4), the incidence of mutation was 1:1200 for Red Elongate and 1:2000 for Bushy Dwarf. Red Elongate appeared only twice in all of the cultures from Bushy Dwarf selfed, a total of 1634 plants. Bushy Dwarf, on the other hand, arose five times among 2173 plants, the progeny of Red Elongate selfed. Tables 1 and 2 record the occurrence of the haploid, Pointed Tips, six times in cultures of selfed Red Elongate (1:200) and twice in the cultures of Bushy Dwarf selfed (1:600).

3. When Bushy Dwarf and Red Elongate were pollinated by *franciscana,* the former appeared as 8.4 per cent of the total and the latter as 3.4 per cent (tables 3 and 4). Only one plant of each sport (culture 31.38, table 3; culture 31.43, table 3, 4) occurred in cultures in which *franciscana* had been pollinated by Red Elongate and Bushy Dwarf, respectively. These probably resulted, not from the fertilization of an *n-franciscana* egg by an $n + 1$ male gamete, but from the fertilization of an $n + 1$ egg by a normal male gamete. The $n + 1$ female gamete probably arose by non-disjunction during megasporogenesis in the *franciscana* parent.

4. Each variant exhibits from 43 to 58 per cent shriveled pollen. There are no giant pollen grains in the pollen, but 4-lobed ones somewhat larger than the normal grains are found. These grains later shrivel, and it is suggested that they carry the 8 chromosomes.

5. Each variant gave rise to forms differing from the parents. There were dwarfs which did not mature and three new types which matured but which have not yet been studied.

6. A cytological study of the variants revealed similar behavior on the part of the two sports. In microsporogenesis a study of the earliest phases

showed a resting nucleus with a delicate thread system, in which were found a variable number of chromatic bodies. The contention of Leliveld (1931) that they represent pairing bodies was not substantiated.

7. The looping of threads between the hollow spireme stage and second contraction is very conspicuous. The loops present at second contraction are seven in number. Six are interpreted as consisting of pairs of chromosomes and the seventh as a trisome.

8. Diakinesis, metaphase, and anaphase figures give the count of fifteen as the chromosome number. The odd chromosome appears either as a univalent or as a member of a trisome, the trisome being a chain. The univalent has its spindle fiber connection either at mid-section or near-terminal. As a rule those of the former type are the ones which lag at heterotypic division. Lagging is common at both divisions, so that as a result very few 8-chromosome microspores are formed. The distribution at the heterotypic division of microsporogenesis is 8 and 7, no 9 and 6 distribution having been observed.

9. In megasporogenesis the meiotic process parallels that in microsporogenesis, except that lagging is less frequent and a 9 and 6 distribution of chromosomes occasionally occurs. Four types of megaspore tetrads are formed, ranging from those with thin walls to those with thick walls. In Red Elongate the chalazal megaspore seemed to be favored over the micropylar one, but in Bushy Dwarf each has about the same chance of development.

10. The $n + 1$ megaspores are not formed as numerously as expected. Their deficiency is largely due to lagging of chromosomes, thus preventing the formation of this type of megaspore. There may in addition be some selective action against the $n + 1$ gamete and the $2n + 1$ zygote. The presence of the $n + 1$ female gamete is probably explained by the much less pronounced lagging of chromosomes in megasporogenesis associated with a smaller degree of environmental maladjustment.

11. The variants Red Elongate and Bushy Dwarf correspond to primaries in *Datura*. There are certain peculiarities of behavior, as, for example, the greater degree of sterility, in which the *Oenothera* and *Datura* sports differ; but this condition may be the result of greater nuclear disturbance occasioned by the addition of an odd chromosome to a haploid set of seven in *Oenothera*, as contrasted with the addition of an odd chromosome to a basic set of twelve in *Datura*.

12. The meiotic behavior in the two variants is interpreted as parasynaptic.

The investigation was carried on under the direction of Professor B. M. Davis, who suggested the problem, furnished certain material from his garden, and gave permission to incorporate from his records such data as were necessary for a complete history of the forms studied. The friendly

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DESCRIPTION OF PLATES

All figures, unless otherwise noted, were sketched 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 20 ×, giving a magnification in the field of the microscope of 2400 diameters. Figures have been reduced to one-half in reproduction.

Plate 22

Figures 1–9. Somatic mitosis in Red Elongate. Fig. 1. The resting nucleus with chromatic bodies. Fig. 2, 3. Early prophase showing rapid thickening of the thread. Fig. 4. Segmenting spireme. Fig. 5. Differentiation of fifteen chromosomes some of

which show lengthwise fission. Fig. 6. Equatorial plate, view from the pole of the spindle. Fig. 7. Early telophase. Fig. 8. Organization of daughter nuclei with chromosomes still in evidence. Fig. 9. Daughter nuclei with chromatic bodies.

Figures 10–27. Microsporogenesis in Red Elongate. Fig. 10–12. Resting nuclei of microsporocytes with chromatic bodies and a delicate thread system. Fig. 13, 14. Early prophase stages showing material passing from the chromatic bodies into a thread system. Fig. 15–17. Prophase stages illustrating a fairly uniform thread system bearing granules. Fig. 18. Presynizesis figure illustrating the thickening of some threads at the expense of others, the threads, however, still thin. Fig. 19. Mid-synizesis. Fig. 20–22. Thickening of the threads and shortening of the thread system after emergence from synizesis. The hollow spireme is exhibited in figure 21. Fig. 23–26. Conspicuous looping during the stages immediately before second contraction. Fig. 27. Second contraction.

Plate 23

Figures 28–45. Microsporogenesis in Red Elongate. Fig. 28, 29. Emergence from second contraction. Fig. 30, 31. Diakinesis, 30 *a*, an open ring from which will come the chromosome arrangements *a* of figure 33. 30 *b*, a closed ring which at metaphase gives rise to a shield-shaped pair as *b* of figure 33. Fig. 32. Multipolar spindle. Fig. 33. Metaphase showing at the right a trisome. *a*, a dumbbell-shaped pair derived from an open ring as *a* of figure 30. *b*, a shield-shaped pair derived from a closed ring as in *b* of figure 30. Fig. 34. Anaphase with one lagging chromosome. Fig. 35. Polar view of late anaphase, the chromosomes of which show no split. Fig. 36, 37. Telophases. A 7–8 distribution of chromosomes. Some indications of lengthwise splitting. Fig. 38–40. Interkinesis with split chromosomes. Fig. 41. Later interkinesis, the chromosomes forming a simple thread system. Fig. 42, 43. Homeotypic metaphases with 8 and 7 split chromosomes, respectively. Fig. 44. Homeotypic mitosis. Early anaphase in one figure and a polar view of metaphase in the other. Fig. 45. Lagging at homeotypic anaphase.

Plate 24

Fig. 46. Microsporogenesis in Red Elongate. Tetrad. Development of chromatin network through elongation and anastomosing of the chromosomes. × 160.

Figures 47–63. Megasporogenesis in Red Elongate. Fig. 47. Resting nucleus, chromatic bodies. Fig. 48. Early prophase with delicate thread system. Fig. 49. Mid-synizesis. Fig. 50, 51. Thicker thread system emerging from synizesis. Fig. 52. Thick spireme following synizesis. Fig. 53, 54. Approaching second contraction. Endonucleoli shown in figure 53. Fig. 55, 56. Second contraction illustrating loops, usually seven in number. Fig. 57, 58. Diakinesis with closed and open rings. Fig. 59. Heterotypic metaphase with the univalent and seven pairs of chromosomes. Fig. 60. Heterotypic metaphase with trivalent and six pairs of chromosomes. Fig. 61. Early anaphase with fifteen chromosomes. Fig. 62. Late anaphase showing 7–8 distribution of chromosomes. Fig. 63. Early telophase showing a 9–6 distribution of chromosomes, no splitting in evidence.

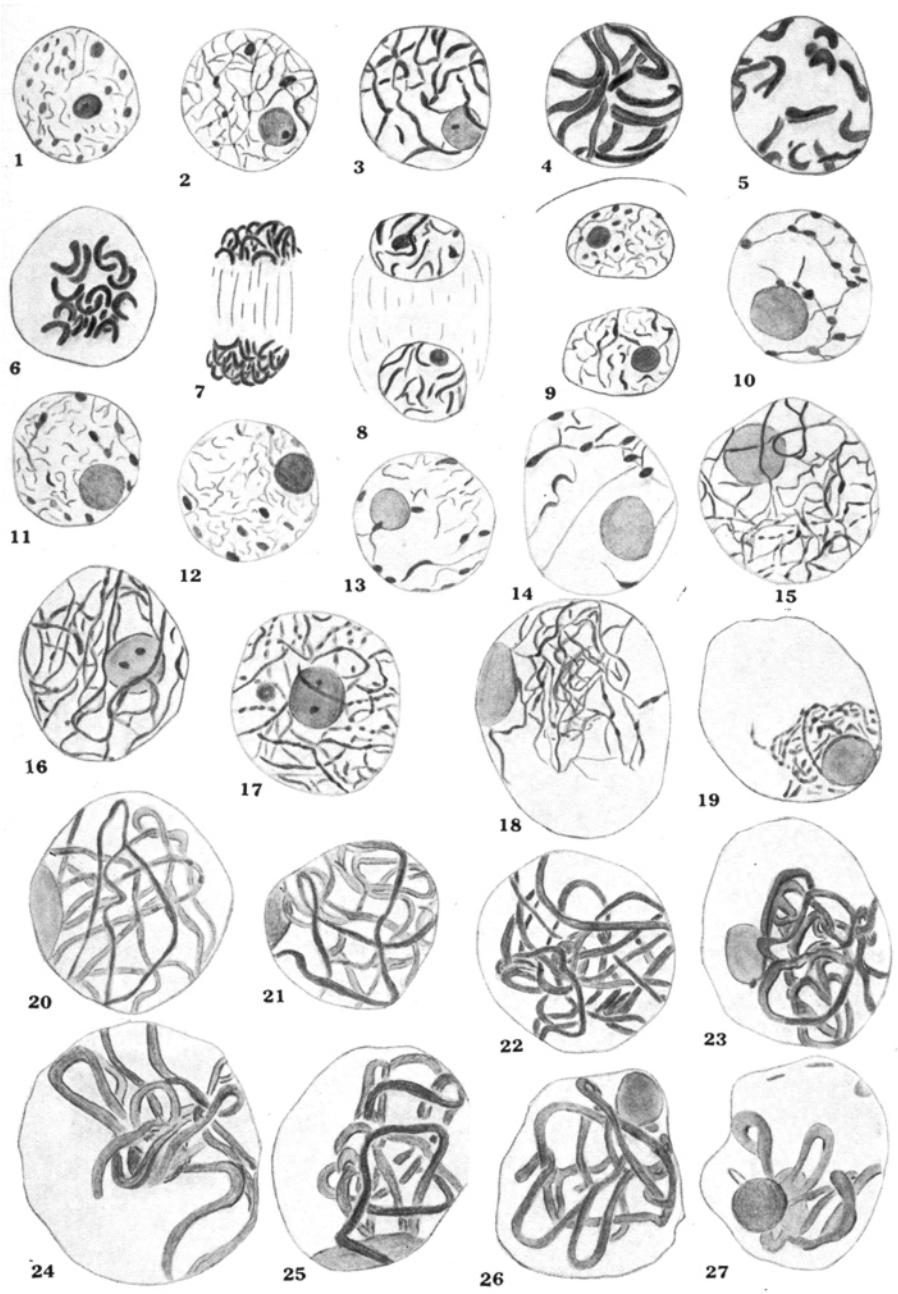
Plate 25

Figures 64–73. Megasporogenesis in Red Elongate. Fig. 64. Late telophase illustrating a 7–7 distribution, chromosomes split. Fig. 65. Interkinesis illustrating 8–7 distribution. Fig. 66. Homeotypic metaphase. Fig. 67. Homeotypic anaphase with 14 and 16 chromosomes. Fig. 68. Homeotypic telophase illustrating lagging of one chromosome. Fig. 69–73. Types of tetrads formed as a result of homeotypic division, showing varying thicknesses of the cell walls. × 160.

PLATE 26

Figures 74–75. Megasporogenesis in Red Elongate. Fig. 74. Megaspore. × 1200. Fig. 75. Female gametophyte. *s*, synergids, with vacuoles below. *e*, egg. *p*, polar nucleus. × 320.

Figures 76–89. Microsporogenesis in Bushy Dwarf. Fig. 76. Resting nucleus in microsporocyte, chromatic bodies. Fig. 77. Early prophase, formation of delicate thread system. Fig. 78. Mid-synizesis. Fig. 79. Hollow spireme following synizesis. Fig. 80. Second contraction showing seven loops. Fig. 81. Diakinesis with six rings and a trisome. Fig. 82. Heterotypic metaphase. Fig. 83. Early anaphase. Fig. 84. Late anaphase with a lagging chromosome. Fig. 85. Interkinesis, 7 and 8 split chromosomes. Fig. 86. Homeotypic metaphase divisions, an 8–7 distribution of chromosomes. Fig. 87. Normal 3-lobed pollen and two 3-lobed shriveled grains. × 80. Fig. 88. 4-lobed pollen grains believed to carry eight chromosomes. × 80. Fig. 89. Older 4-lobed grains now shriveled. × 80.



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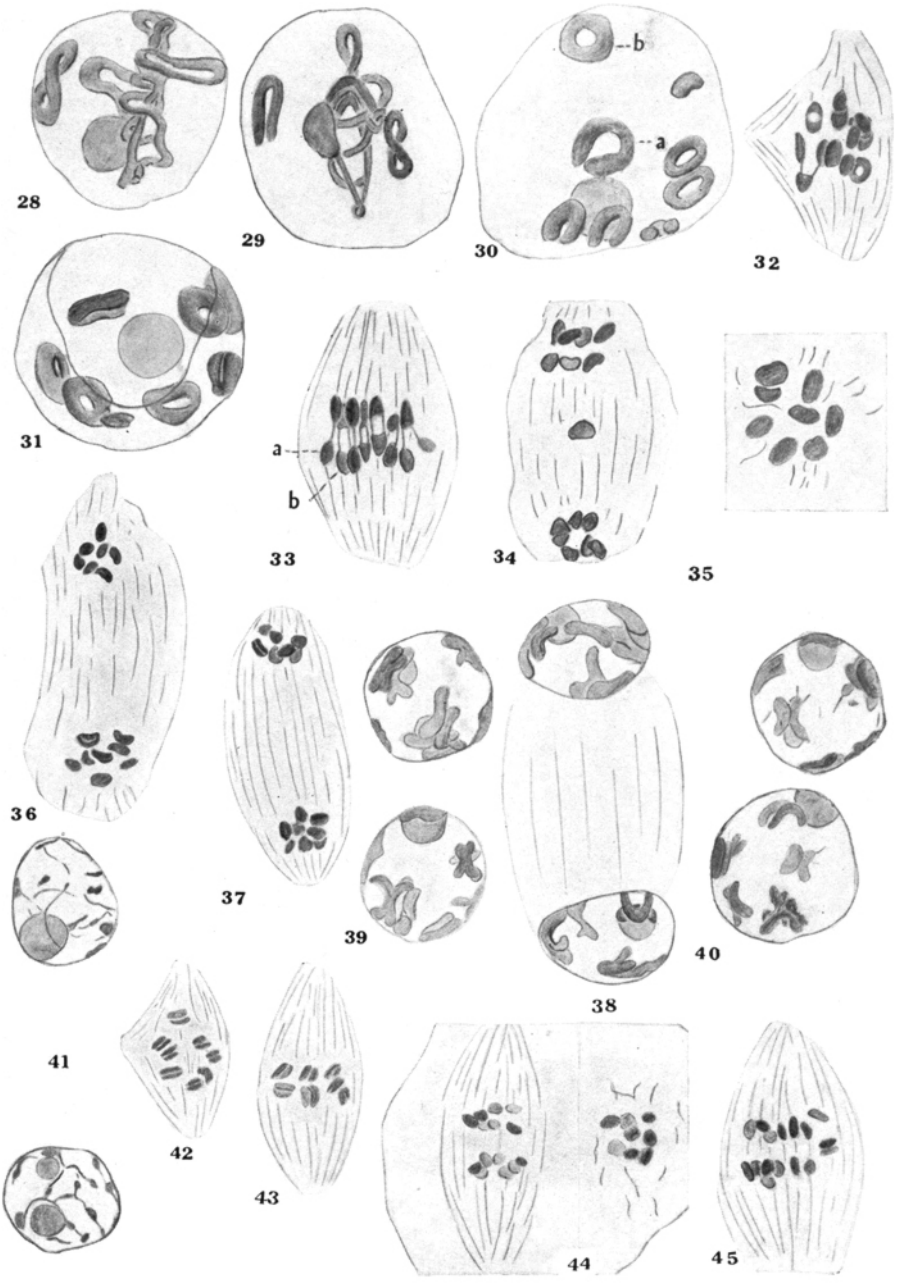

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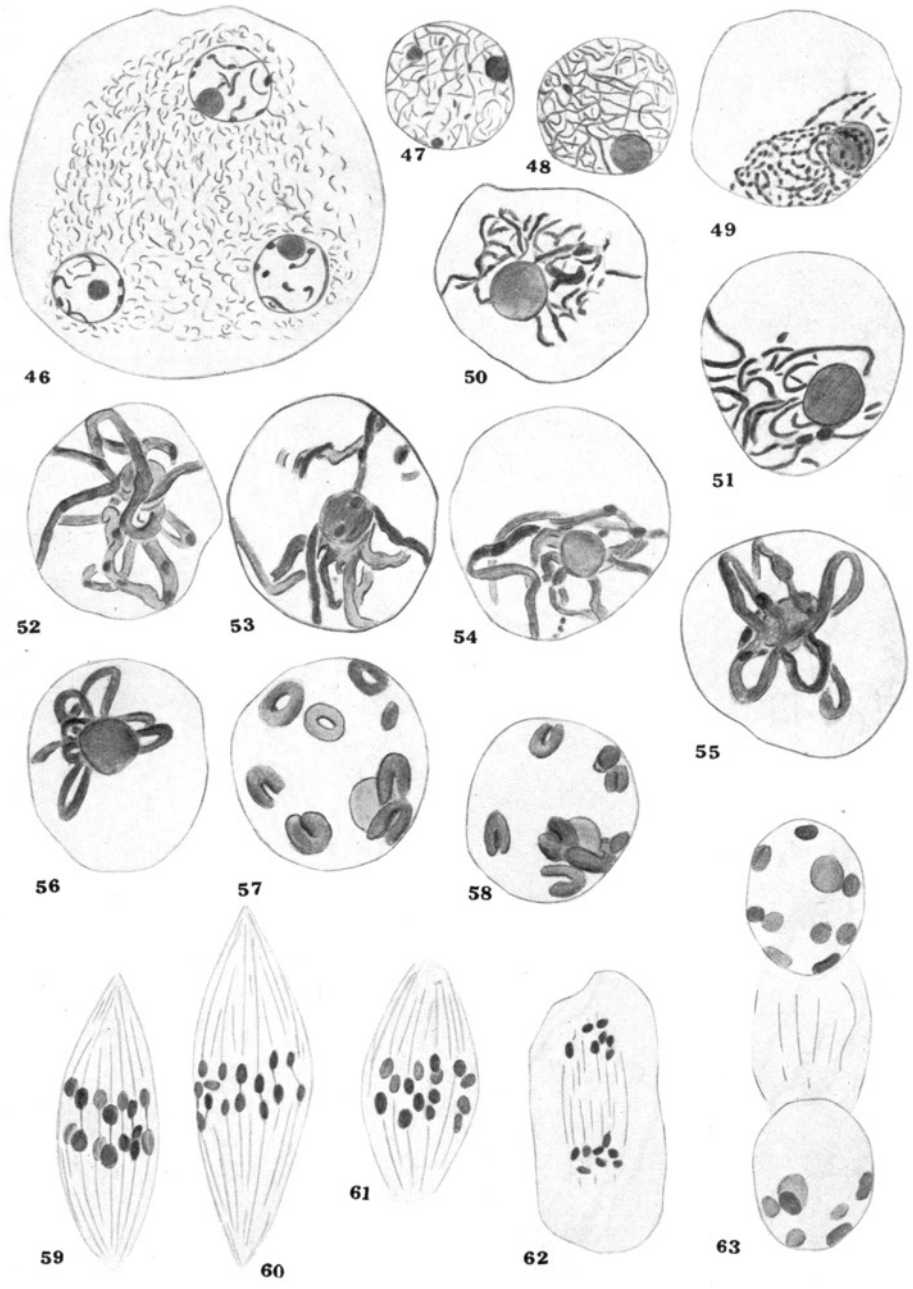

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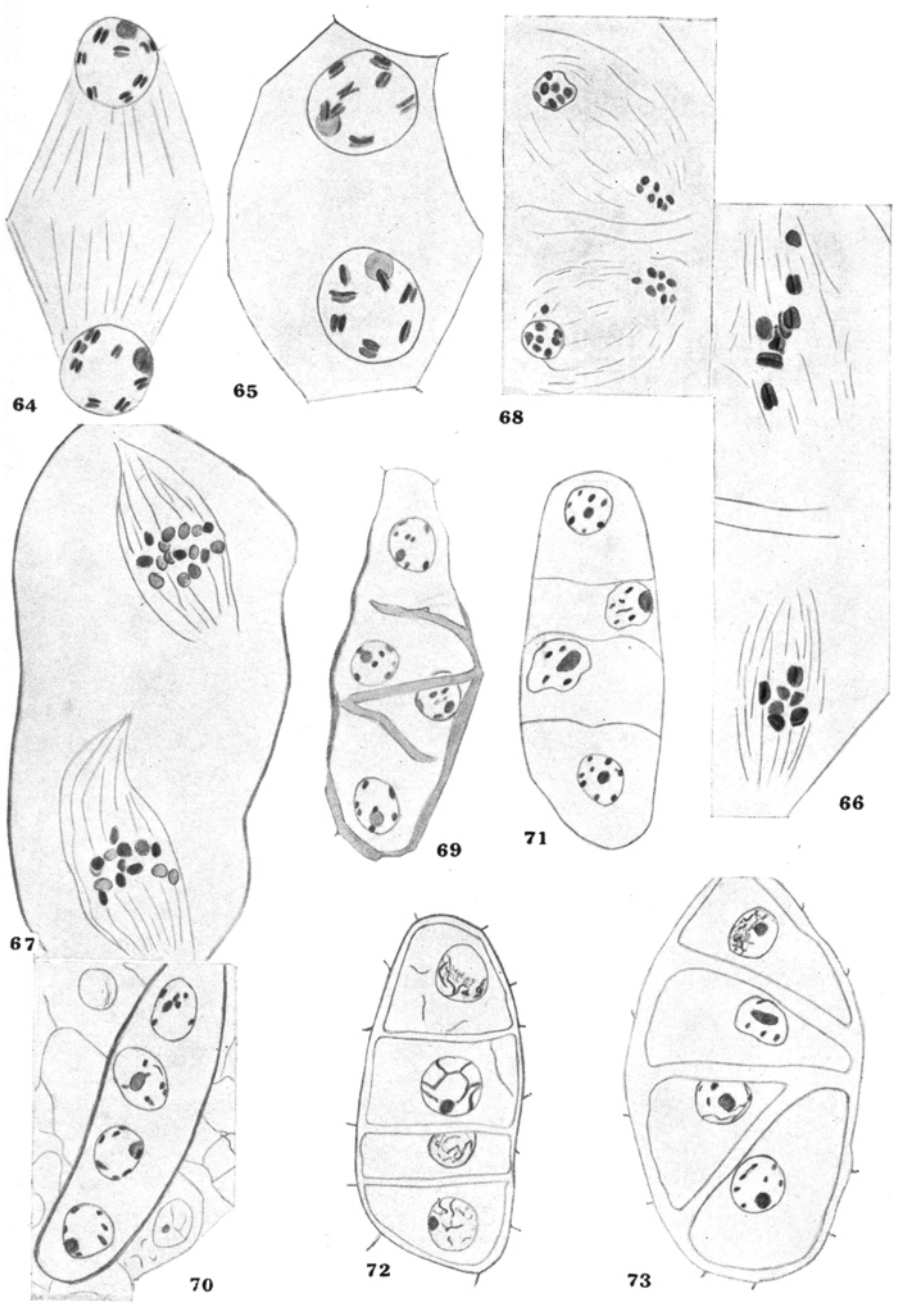

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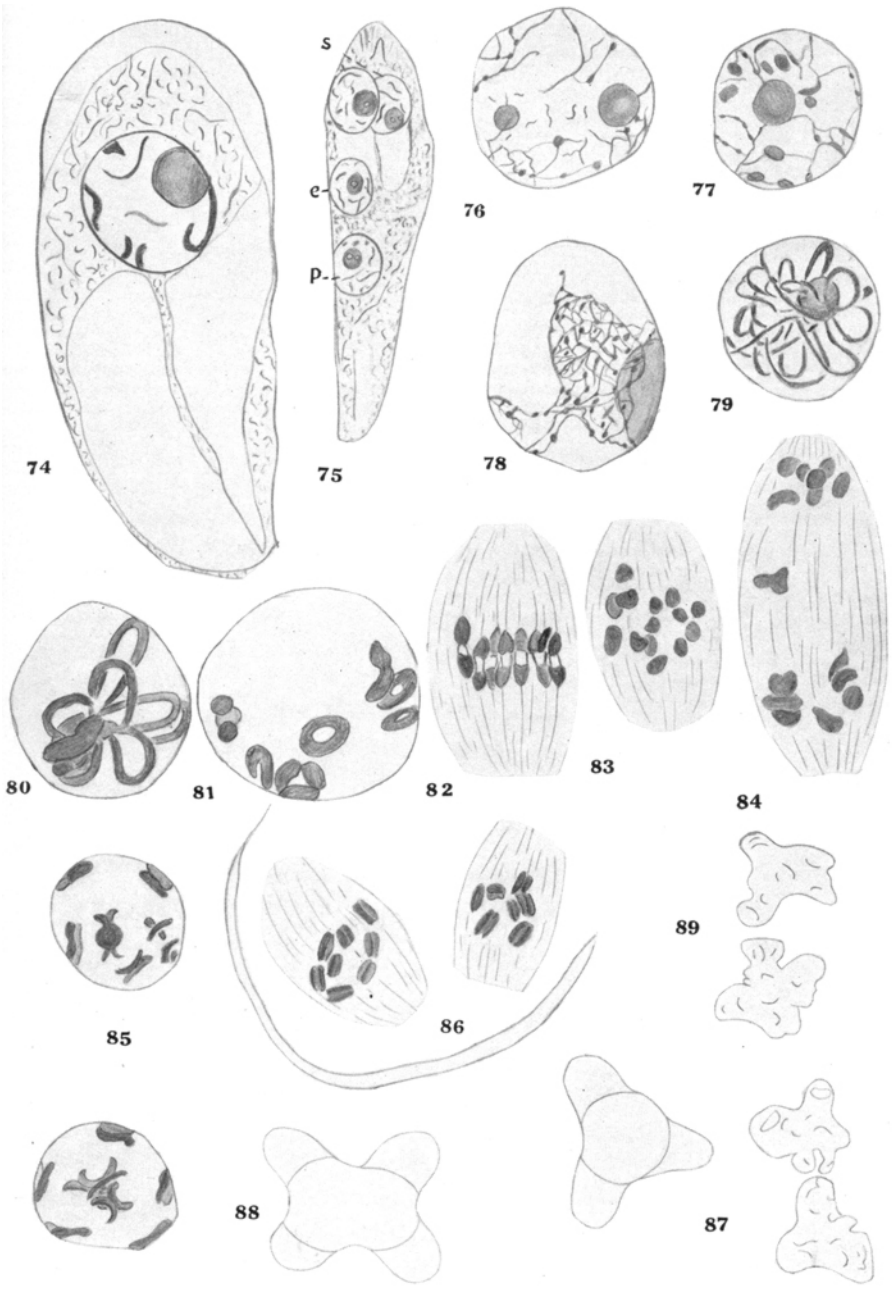

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